

THE ECOLOGY OF BENTHOPELAGIC FISH AT OFFSHORE WIND FARMS
Towards an integrated management approach

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THE ECOLOGY OF BENTHOPELAGIC FISH AT OFFSHORE WIND FARMS
Towards an integrated management approach

DE ECOLOGIE VAN BENTHOPELAGISCHE VIS BIJ OFFSHORE WINDMOLENS
Naar een geïntegreerd beheer van de zee

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(Marine Sciences)

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Summary

The use of wind power by mankind has a long history and dates back about 3000 years in time. For an extended period windmills were mainly used for grinding grain and pumping water and it lasted until 1891 before the first electricity generating wind turbine was constructed. By the end of the 1990s, wind energy production had become one of the most important renewable energy resources in the world. Offshore wind farms on the other hand are a relatively new concept. The first large scale offshore wind farm in the world was built in 2000 off the coast of Denmark and from that time onwards offshore wind power development expanded rapidly. All across the North Sea wind farms are planned, under construction and operational. Thousands of wind turbines will be present and as a result new hard substrate habitats, through the wind turbine foundations, arise. In the Belgian part of the North Sea, the wind turbine foundations form artificial reefs in a marine environment formerly dominated by a sandy seabed. These artificial reefs, the so-called windmill artificial reefs (WARs) influence the ecosystem functioning and the local biodiversity; and interactions within and between the reef and the surrounding soft substrate habitat will occur.

In this study, we focused on the reef effects influencing benthopelagic fish in the Southern North Sea. It is known that (windmill) artificial reefs attract and concentrate fishes. However, whether the fishes are merely attracted or if production or an ecological trap occurs is difficult to unravel. In case of attraction, the fish move from the surrounding environment towards the reef. They aggregate at the reef, but there is no net increase in the local population. If production occurs, the carrying capacity of the environment increases as a result of the new habitat. More fish are able to settle, survive, grow and contribute to the local population. The fish can also be caught in an ecological trap, if they are attracted to, and preferably settle in a habitat with suboptimal conditions relative to other available habitats. A set of questions related to fish community structure, behavioural ecology and reef mechanisms involved in fish production in the specific environment need to be answered to unravel the issue. Based on the outcome of the issue we also discussed whether small-scale fisheries should be allowed inside the offshore wind farms.

From 2009 until 2012 we investigated the attraction-production hypothesis for dominant fish species related to the WARs. Information on length-frequency distribution, diet, community structure and movements of Atlantic cod (*Gadus morhua*) and pouting (*Trisopterus luscus*) was gathered in an offshore wind farm in the Belgian part of the North Sea. A multitude of techniques (i.e. visual observations with divers, hand line sampling campaigns, acoustic telemetry and stomach content analyses) were applied and integrated to gain insights on their behavioural ecology and to unravel whether production occurs at the WARs.

We found that both Atlantic cod and pouting are strongly attracted towards the WARs. Much higher average catch rates were recorded at the WARs in comparison to the reference areas. For Atlantic cod average catch per unit effort was 4.6 ± 0.9 ind $h^{-1} fm^{-1}$ at the WARs, while it was 0.1 ± 0.03 and 1.1 ± 0.2 ind $h^{-1} fm^{-1}$ for the sandy areas and wrecks respectively. For pouting it was 4.3 ± 0.6 , 0.1 ± 0.03 and 0.7 ± 0.1 ind $h^{-1} fm^{-1}$ at the WARs, sandy areas and wrecks respectively.

A more detailed investigation of the community structure of both species revealed that especially younger age groups of both species are attracted towards the WARs. For Atlantic cod mainly age group I and II were encountered, while for pouting it was age group 0 and I. The fish are not present throughout the year. There is a clear seasonal pattern in aggregation behaviour. The highest numbers of fish were noted during summer and autumn (with a mean monthly catch rate of up to 13.4 and 12.8 ind $h^{-1} fm^{-1}$ for Atlantic cod and pouting respectively). In winter time almost no individuals were encountered. Probably movements related to spawning explain the seasonality in presence at the WARs.

Further, we demonstrated that, during the period they were present near the WARs, Atlantic cod exhibited strong residency and high site fidelity. Most of the tagged fish were present on a daily basis for 75% of the time of the monitoring period.

Stomach content analyses revealed that both Atlantic cod and pouting fed on the epifaunal species present at the WARs. The dominant prey species in the diet of pouting were *Jassa herdmani*, *Pisidia longicornis*, *Pisces* sp. and *Liocarcinus* spp. In the diet of Atlantic cod *J. herdmani*, *P. longicornis*, *Liocarcinus* spp., *Necora puber*, and *Pisces* sp. were most dominant. Some amphipod species (i.e. *Phtisica marina* and *Monocorophium acherusicum*) had a high frequency of occurrence as well and reached high abundances, but contributed less to the total prey biomass for both species. The predominant prey species in the diet were all present in high densities at the WARs.

To acquire more information on the quality of the food, energy profiling of both fish species was performed. The fishes had more energy available than required to maintain their metabolism. Thus, enough energy was left for growth and reproduction. As a result the WARs are considered a suitable feeding ground with sufficient, good quality food available. In addition, the fitness of pouting and Atlantic cod was compared between the WARs and the reference areas. No significant differences in fitness were found, indicating the WARs are not inferior in quality to the reference habitats. Based on the integrated results it was concluded that production occurs on a local scale (i.e. at the WARs). However, so far no changes in productivity were observed on a regional scale.

The results obtained during this study allowed to describe the life-history of Atlantic cod and pouting at the WARs. The age group I Atlantic cod arrive at the WARs in April-May. They feed on the epifaunal prey species present, grow and stay in the area until the end of the year. By winter most I-group individuals have left the WARs and only few specimens come back after

the spawning period. For pouting the 0-group arrives at the WARS in September and feeds on the epifaunal prey species. They leave the area by January but by May the I-group is back at the WARS and stay again until the end of the year. During this period feeding and growth are observed.

The offshore wind farms in the Belgian part of the North Sea are closed to fisheries. However, pressure groups aiming at the facilitation of passive fisheries inside the wind farm concession areas, are active in Belgium. Based on the current knowledge on the ecology and population structuring of Atlantic cod and pouting at the WARS, we conclude that no fisheries activities should be allowed inside the offshore wind farms in the Belgian part of the North Sea. We support this statement with several arguments: 1) no indication of regional production was observed yet; 2) juvenile fish dominated the catches; 3) there is a seasonal pattern in presence and 4) fisheries exclusion areas will benefit both fish populations and fisheries.

In conclusion, we demonstrated that WARS influence the behavioural ecology of Atlantic cod and pouting. They benefit from these artificial hard substrates and thrive well in this environment closed to fisheries. We support this fisheries closure, because the benefits are exported beyond the boundaries of the wind farm concession since the fish leave the protective area once they grow older. Proper management, through well-thought-out marine spatial planning and regulations, should be implemented to reduce conflicts and use the marine resources in a sustainable way.

Samenvatting

Het gebruik van windenergie door de mens kent een lange geschiedenis en gaat meer dan 3000 jaar terug in de tijd. Gedurende een lange periode werden windmolens voornamelijk gebruikt om graan te malen en water op te pompen en het duurde tot 1891 voordat de eerste windmolen werd gebouwd die elektriciteit produceerde. Tegen het einde van de jaren 1990 was windenergieproductie wereldwijd één van de belangrijkste bronnen van hernieuwbare energie. Offshore windmolenparken daarentegen zijn een relatief nieuw concept. Het eerste grootschalige park werd gebouwd in het jaar 2000 voor de kust van Denemarken. Vanaf dat moment is offshore windenergieproductie sterk uitgebreid. In de ganse Noordzee zijn er windmolenparken gepland, onder constructie of reeds operationeel. In de nabije toekomst zullen duizenden windmolens aanwezig zijn en als gevolg hiervan zullen overal in de Noordzee nieuwe hard-substraat habitats verschijnen. De funderingen van de windmolens in het Belgisch deel van de Noordzee vormen artificiële riffen in een milieu dat voorheen gedomineerd werd door een zandige zeebodem. Deze artificiële riffen, de zogenaamde windmolen artificiële riffen (WARs), beïnvloeden het ecosysteemfunctioneren en de lokale biodiversiteit. Ook interacties binnen en tussen het rif en de omliggende zacht-substraat habitats zullen plaatsvinden.

In deze studie werd de focus gelegd op de rifeffecten die van belang zijn voor benthopelagische vis. Het is algemeen geweten dat (windmolen) artificiële riffen vissen aantrekken en concentreren op één locatie. Het is echter niet gemakkelijk om te ontwarren of er enkel attractie plaatsvindt of dat ook productie of een ecologische val plaatsvinden. Indien er attractie plaatsvindt, verplaatsen de vissen zich van de omliggende omgeving naar het rif. Ze aggregeren aan het rif, maar er is geen toename in de lokale populatie. Bij productie neemt de draagkracht van het systeem toe als gevolg van het nieuwe habitat. Meer vissen hebben de mogelijkheid om zich te vestigen, te overleven, te groeien en toe te dragen aan de lokale populatie. De vissen kunnen echter ook in een ecologische val terecht komen. Dit gebeurt indien ze aangetrokken zijn tot, en zich bij voorkeur vestigen in een habitat met suboptimale condities in vergelijking tot andere beschikbare habitats. Het is niet gemakkelijk om de verschillende situaties van elkaar te onderscheiden. Een reeks vragen, gerelateerd aan de gemeenschapsstructuur, het gedrag en de rifeffecten die betrokken zijn bij de visproductie moeten beantwoord worden om de situatie te ontwarren. De uitkomst van de situatie is gekoppeld aan de vraag of kleinschalige visserij toegelaten moet worden binnen de windmolenparken.

Tussen 2009 en 2012 werd de attractie-productie hypothese onderzocht voor dominante vissoorten aan de WARs. Er werd informatie verzameld over lengte-frequentieverdelingen, het dieet, de gemeenschapssamenstelling en de bewegingen van kabeljauw (*Gadus morhua*)

en steenbolk (*Trisopterus luscus*) in een windmolenpark in het Belgisch deel van de Noordzee. Dit om inzicht te verkrijgen in de gedragsecologie van beide soorten en om na te gaan of productie plaatsvindt binnen de WARs. Verschillende technieken (zijnde visuele observaties met duikers, handlijnvisserij, akoestische telemetrie en maaganalyses) werden toegepast en geïntegreerd om de vragen te kunnen beantwoorden.

Er werd waargenomen dat zowel kabeljauw als steenbolk aangetrokken zijn tot de WARs. Veel hogere gemiddelde vangstefficiënties werden waargenomen aan de WARs in vergelijking met de referentiegebieden. Voor kabeljauw werd een gemiddelde vangstefficiëntie van $4.6 \pm 0.9 \text{ ind h}^{-1} \text{ fm}^{-1}$ genoteerd aan de WARs, terwijl dit respectievelijk slechts 0.1 ± 0.03 en $1.1 \pm 0.2 \text{ ind h}^{-1} \text{ fm}^{-1}$ was aan de zandige gebieden en de scheepswrakken. Voor steenbolk was dit respectievelijk 4.3 ± 0.6 , 0.1 ± 0.03 en $0.7 \pm 0.1 \text{ ind h}^{-1} \text{ fm}^{-1}$ aan de WARs, zandige gebieden en scheepswrakken.

Gedetailleerde informatie over de gemeenschapssamenstelling van beide soorten toonde aan dat specifieke leeftijdsgroepen aangetrokken zijn tot de WARs. Voor kabeljauw zijn dit voornamelijk de leeftijdsgroepen I en II, terwijl dit de 0 en I groep zijn voor steenbolk. Hieruit kunnen we besluiten dat voornamelijk jongere leeftijdsgroepen aangetrokken zijn tot de WARs.

De vissen zijn echter niet het ganse jaar aanwezig, er is een duidelijk seizoenaal patroon in aggregatiegedrag. De hoogste aantallen vis worden waargenomen (via duiken), gevangen (via lijnvisserij) of gedetecteerd (via telemetrie) tijdens de zomer en het najaar (er werd een gemiddelde vangstefficiëntie tot 13.4 en 12.8 $\text{ind h}^{-1} \text{ fm}^{-1}$ waargenomen voor respectievelijk kabeljauw en steenbolk). In de winter worden nauwelijks nog individuen waargenomen. Deze seizoenaliteit kan waarschijnlijk verklaard worden door voortplantingsmigraties.

Verder werd er aangetoond dat, tijdens de periode dat kabeljauw aanwezig is, deze een hoge residentie en plaatsgetrouwheid vertonen. De meeste gezenderde vissen werden op een dagelijkse basis gedetecteerd voor 75% van de tijd van de monitoringsperiode.

Maaganalyses bewezen dat zowel kabeljauw als steenbolk zich voeden met de epifauna aanwezig aan de WARs. De dominante prooisorten in het dieet van steenbolk waren *Jassa herdmani*, *Pisidia longicornis*, *Pisces* sp. en *Liocarcinus* spp. In het dieet van kabeljauw waren *J. herdmani*, *P. longicornis*, *Liocarcinus* spp., *Necora puber*, en *Pisces* sp. dominant. Sommige soorten amfipoden (zijnde *Phtisica marina* en *Monocorophium acherusicum*) werden veel waargenomen in hoge aantallen, maar droegen minder bij tot de totale biomassa van de prooisorten. De dominante prooisorten in het dieet zijn ook in hoge densiteiten aanwezig aan de WARs.

Om meer informatie te bekomen over de kwaliteit van het opgenomen voedsel, werd een 'energy profiling' uitgevoerd voor kabeljauw en steenbolk. Beide hadden meer energie beschikbaar dan nodig om hun basaal metabolisme te onderhouden. Dus er was genoeg energie beschikbaar voor groei en reproductie. Als gevolg hiervan worden de WARs

beschouwd als een geschikte voedingsbodem met voldoende voedsel van goede kwaliteit. Daarnaast werd de fitness van kabeljauw en steenbolk vergeleken tussen de WARs en de referentiegebieden. Er werden geen significante verschillen in fitness waargenomen, waaruit besloten kan worden dat de WARs niet minderwaardig zijn in kwaliteit dan de referentiegebieden. Gebaseerd op de geïntegreerde resultaten werd er geconcludeerd dat er productie plaatsvindt op lokale schaal (dus aan de WARs). Er is echter tot dusver geen bewijs voor extra productie op regionale schaal.

De resultaten verkregen gedurende deze studie laten toe de levensgeschiedenis van kabeljauw en steenbolk aan de WARs te schetsen. De kabeljauwen van leeftijdsgroep I komen toe aan de WARs in april-mei. Ze voeden zich met de aanwezige epifauna, groeien en blijven in het gebied tot het einde van het jaar. Tegen de winter hebben de meeste individuen het gebied verlaten en slechts enkele individuen komen terug na de voortplantingsperiode. Bij steenbolk, arriveert de 0-groep aan de WARs in september en voeden zich met de aanwezige epifauna. Ze verlaten het gebied tegen januari, maar rond mei keren ze terug naar de WARs en blijven tot het einde van het jaar. Gedurende deze periode wordt voeding en groei waargenomen.

De offshore windmolenparken in het Belgisch deel van de Noordzee zijn tot op heden gesloten voor visserij. Verschillende belangengroepen ijveren echter voor een regularisatie van passieve visserij binnen de windmolenparken. Op basis van de huidige kennis van de ecologie en de populatiestructurering van kabeljauw en steenbolk aan de WARs werd er geconcludeerd dat er geen visserijactiviteiten moeten worden toegelaten binnen offshore windmolenparken in het Belgisch deel van de Noordzee. Verschillende argumenten liggen hiervoor aan de basis: 1) er is geen indicatie van regionale productie, 2) juveniele vissen domineren de vangsten, 3) er is seizoensaliteit in aanwezigheid, 4) zowel de vispopulaties als visserij hebben baat bij visserijvrije zones.

We kunnen concluderen dat de WARs de gedragsecologie van kabeljauw en steenbolk beïnvloeden. Ze hebben baat bij deze artificiële riffen en doen het goed in het gesloten gebied. Wij steunen het visserijverbod en zijn ervan overtuigd dat de voordelen geëxporteerd worden tot buiten de grenzen van het windmolenpark, aangezien de vissen het beschermde gebied verlaten van zodra ze ouder zijn. Een passend beheer, via weloverdachte mariene ruimtelijke planning en via regularisaties, zou moeten worden toegepast om conflicten in te perken en de mariene bronnen op een duurzame manier te gebruiken.

Niet-technische samenvatting

Het gebruik van windenergie door de mens kent een lange geschiedenis en gaat meer dan 3000 jaar terug in de tijd. Tegenwoordig zijn windmolens, als duurzame energiebron, niet meer weg te denken uit het Europese landschap en intussen worden ook op zee windmolens gebouwd. Op zee is er veel ruimte en het waait er harder en regelmatig dan op land, waardoor de energie-opbrengst verhoogt. Het eerste grootschalige offshore windmolenpark werd gebouwd in het jaar 2000 voor de kust van Denemarken. Intussen zijn er in de ganse Noordzee windmolenparken gepland, onder constructie of reeds operationeel.

De funderingen van deze windmolens vormen kunstmatige riffen die een invloed uitoefenen op de lokale soortenrijkdom en op het functioneren van het ecosysteem. De soortensamenstelling van zowel vogels, zeezoogdieren, vissen, bodemorganismen en organismen die leven op en rond de funderingen zijn duidelijk verschillend in vergelijking met de controlezones (zonder windmolens, maar met een vergelijkbaar habitat). In deze studie spitsen we ons toe op de vissen en worden de gemeenschapsstructuur, het gedrag van de vissen en mogelijke rifeffecten die betrokken zijn bij visproductie onderzocht. Op basis van de resultaten van deze studie wordt nagegaan of visserij toegelaten kan worden binnen de windmolenparken.

Tussen 2009 en 2012 werd de ecologie van vissen in een windmolenpark in het Belgisch deel van de Noordzee onderzocht, met speciale aandacht voor kabeljauw en steenbolk. Er werd informatie verzameld over lengte-frequentie verdelingen, het dieet, de gemeenschapssamenstelling en de migratiepatronen van deze soorten. Deze waarnemingen werden uitgevoerd op basis van wetenschappelijk duiken, handlijn-visserij, akoestische telemetrie en maaganalyses.

Een eerste deel van het onderzoek wijst uit dat zowel kabeljauw als steenbolk aangetrokken zijn tot de windmolenparken. Ze komen er voor in hoge aantallen (met een gemiddelde vangstefficiëntie tot 13.4 en 12.8 ind $h^{-1} fm^{-1}$ voor respectievelijk kabeljauw en steenbolk) en het zijn voornamelijk de jongere leeftijdsgroepen (0 to 2 jaar oud) die er aggregeren. Beide soorten vertonen een seizoenaal patroon in aanwezigheid met verhoogde densiteiten in de zomer en het najaar, terwijl in de winter nauwelijks nog individuen voorkomen. De periode dat kabeljauw aanwezig is rond de windmolens, zijn de individuen zeer plaatsgetrouw en blijven gedurende langere tijd (tot verschillende maanden) rond de windmolens. Hierbij verkiezen ze het harde substraat van de riffen boven het omliggende zachte substraat.

In het tweede deel van de studie wordt nagegaan waarom kabeljauw en steenbolk aangetrokken zijn tot de windmolens en er zo plaatsgetrouw blijven. De resultaten tonen aan dat zowel kabeljauw als steenbolk zich voeden met organismen die leven op de riffen. Een aantal organismen (vlokkreeftje, porseleinkrabbetje en zwemkrab) die talrijk voorkomen

op het rif, zijn ook dominant in hun dieet. Daarenboven is het voedsel van goede kwaliteit en verkeren de vissen die er voorkomen in goede conditie. Verder biedt het rif ook een schuilplaats tegen predatoren.

De resultaten verkregen gedurende deze studie laten toe de levensgeschiedenis van kabeljauw en steenbolk in de buurt van de windmolenparken te schetsen. De kabeljauwen van 1 jaar oud komen toe in de windmolenparken in april-mei. Ze voeden zich met de aanwezige epifauna, groeien en blijven in het gebied tot november-december. Tegen de winter hebben de meeste individuen het gebied verlaten en slechts enkele individuen komen terug na de voortplantingsperiode. Bij steenbolk arriveert de 0-groep in september en voeden zich met de aanwezige epifauna. Ze verlaten het gebied tegen januari, maar rond mei keren ze terug naar de WARs en blijven tot het einde van het jaar. Gedurende deze periode wordt voeding en groei waargenomen.

We kunnen besluiten dat er productie van kabeljauw en steenbolk plaatsvindt in de windmolenparken (de vissen voeden er zich, groeien en verkeren in goede conditie). Er is echter tot dusver geen bewijs voor extra productie op regionale schaal.

De offshore windmolenparken in het Belgisch deel van de Noordzee zijn tot op heden gesloten voor visserij. Verschillende belangengroepen ijveren echter voor een regularisatie van passieve visserij binnen de windmolenparken. Op basis van de huidige kennis van de ecologie van kabeljauw en steenbolk zijn wij van mening dat er geen visserijactiviteiten mogen worden toegelaten binnen offshore windmolenparken in het Belgisch deel van de Noordzee. Verschillende argumenten liggen hiervoor aan de basis: 1) er is geen indicatie van regionale productie, 2) juveniele vissen domineren de populaties in de windmolenparken, 3) de soorten vertonen seizoensaliteit in aanwezigheid en 4) zowel de vispopulaties als visserij hebben baat bij visserijvrije zones, die in dit geval in de windmolenparken kunnen afgebakend worden.

In deze studie hebben we duidelijk vastgesteld dat de gedragsecologie van kabeljauw en steenbolk in de windmolenparken verandert (in vergelijking met omliggende controlegebieden). Deze vissen hebben baat bij deze artificiële riffen en doen het goed in het, voor de visserij, gesloten gebied. Wij steunen het visserijverbod en zijn ervan overtuigd dat de voordelen (betere overlevingskansen, goede conditie en groei) geëxporteerd worden tot buiten de grenzen van het windmolenpark, aangezien de vissen het beschermde gebied verlaten van zodra ze ouder zijn. Een passend beheer, via weloverdachte mariene ruimtelijke planning en regelgeving, zou moeten worden toegepast om conflicten in te perken en de mariene voedselbronnen op een duurzame manier te gebruiken.

List of symbols and abbreviations

Abbreviation	Description	Unit
%A	abundance index	
%FO	frequency of occurrence	
%G	gravimetric index	
%N	numeric index	
AFDW	ashfree dry weight	g
ANOSIM	analysis of similarity	
ANOVA	analysis of variance	
AR	artificial reef	
BPNS	Belgian part of the North Sea	
CEA	cellular energy allocation	
CPUE	catch per unit effort (based on line fishing)	ind h ⁻¹ fm ⁻¹
DF	dilution factor	
DHA	docosahexaenoic acid	
DW	dry weight	g
E _a	energy availability	
E _c	energy consumption	
EPA	eicosapentaenoic acid	
ETS	electron transport system	
FA	fatty acid	
FAME	fatty acid methyl esters	
FFT	fast fourier transformation	
FWO	Flemish Fund for Scientific Research	
GBF	Gravity based foundation	
ICES	International Council for the Exploration of the Sea	
ILVO	Institute for Agricultural and Fisheries Research	
INBO	Research Institute for Nature and Forest	
INT	p-iodonitrotetrazolium violet	
IRI	index of relative importance	
IWT	Agency for Innovation by Science and Technology	
MDS	non-metric multidimensional scaling	
MLWS	mean low water spring	
MUMM	Management Unit of the North Sea Mathematical Models	
MW	MegaWatt	
NR	natural reef	
OWF	offshore wind farm	
PRIMER	Plymouth Routines in Multivariate Ecological Research	
PUFA	polyunsaturated fatty acid	

PVP	polyvinylpyrrolidone	
Q	feeding coefficient	
RBINS	Royal Belgian Institute of Natural Sciences	
RV	research vessel	
SBNS	Southern Bight of the North Sea	
SCUBA	self-contained underwater breathing apparatus	
SD	standard deviation	
SE	standard error	
SIMPER	percentages of similarity	
SL	standard length	cm
TCA	Trichloroacetic acid	
TL	total length	cm
UGent	Ghent University	
V	volume	ml, μ l
VLIZ	Flanders Marine Institute	
WAR	windmill artificial reef	
WGS 84	World Geodetic System dating from 1984	
WW	wet weight	g





Chapter 1

General introduction

Context setting: offshore wind farms worldwide & in the Belgian part of the North Sea

The use of wind power by mankind has a long history and dates back about 3000 years in time. For an extended period windmills were mainly used for grinding grain and pumping water and it lasted until 1891 before the first electricity generating wind turbine was constructed (Ackermann and Söder, 2002; Gipe, 1995; Mathew, 2006). By the end of the 1990s, wind energy production had become one of the most important renewable energy resources in the world (Ackermann and Söder, 2002).

Offshore wind farms on the other hand are a relatively new concept. Middelgrunden, the first large scale offshore wind farm in the world was built in 2000 off the coast of Denmark, near Copenhagen. This farm consisted of 20 wind turbines each with a capacity of 2 megawatt (MW) (Sørensen et al., 2001). Two years later, a much larger wind farm was constructed in Denmark: Horns Rev. This wind farm consists of 80 wind turbines, with a 2 MW capacity each (Hvidt et al., 2006). From that time onwards offshore wind farm (OWF) development expanded rapidly in the North Sea and farms are planned, being constructed or already operational in several Northern European countries (e.g. Denmark, United Kingdom, the Netherlands, Belgium, Sweden and Germany) (Arapogianni et al., 2013; Brabant et al., 2012; Shaw et al., 2002). The interest for offshore wind energy development is also growing outside Europe (Musial and Butterfield, 2004). In North America for example the potential for offshore wind farms is currently being investigated (Breton and Moe, 2009).

In the Belgian part of the North Sea (BPNS) the first wind turbines were constructed in 2008. At present, two wind farms are operational (C-Power and Belwind), one is being constructed (Northwind) and four more domain concessions are granted (Norther, Rentel, Seastar and Mermaid) in the BPNS (Fig. 1). With this rapid increase in wind farm development in the (Belgian) North Sea, questions concerning the environmental impact of such farms are raised. In Belgium, the concessionaires are obligated by the law to facilitate monitoring the possible environmental effects of their wind farm. This monitoring serves two main goals: 1) to enable the authorities to mitigate or even halt the activities in case of extreme damage to the marine environment; 2) to understand and evaluate the impact of offshore wind farms on the different aspects of the marine environment and consequently support the future policy regarding offshore wind farms (Brabant et al., 2012). The monitoring programme, set up and coordinated by the Management Unit of the North Sea Mathematical Models (MUMM), investigates

physical, biological and socio-economical aspects of the marine environment (Brabant et al., 2009). Different aspects of the monitoring are carried out by different institutions, each with their own expertise.

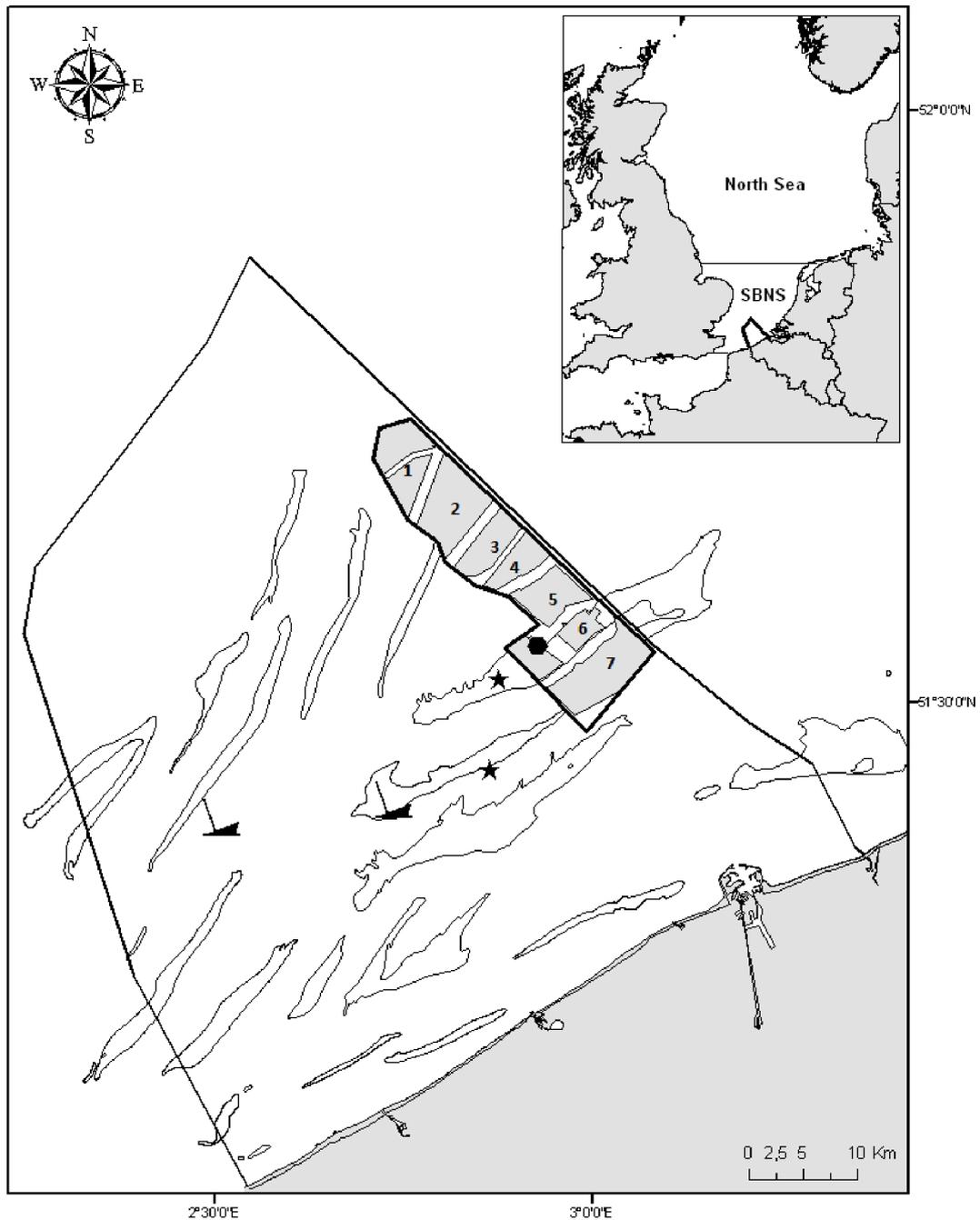


Figure 1. The Belgian part of the North Sea and its position within the Southern Bight of the North Sea (SBNS) and the North Sea. At the East side of the Belgian part of the North Sea a zone is dedicated for renewable energy production. All concession areas have been granted for wind power: 1) Mermaid, 2) Belwind, 3) Seastar, 4) Northwind, 5) Rentel, 6) C-Power and 7) Norther. Sampling locations at the windmill artificial reefs (diamond), sandy areas (stars) and shipwrecks (wrecks) are indicated. The lines represent subtidal sandbanks.

MUMM conducts the studies on underwater noise, hydrodynamics, electromagnetic fields, hard substrate epifauna, marine mammals and seabird radar detections. The Research Institute for Nature and Forest (INBO) investigates the impacts on seabirds, while the Institute for Agricultural and Fisheries Research (ILVO) tackles the effects on the soft substrate epibenthos, fish and underwater noise. The Marine Biology Research Group of Ghent University is responsible to follow-up potential changes in the soft substrate macrobenthos and hard substrate associated fishes. Next to regular monitoring measurements, interesting research questions can be answered in order to mitigate eventual impacts of these new structures in the sea (see further).

Where possible, the Before-After/Control-Impact (BACI) methodological approach was applied for the monitoring (Smith et al., 1993). The BACI design aims to investigate the possible changes in the environment before and after the wind farm was built. In addition, changes are compared between a reference site and the actual impact area. The reference site should be highly comparable to the impact area in abiotic conditions (i.e. similar hydrodynamic conditions, depth range, granulometry).

However, for some environmental assets (investigation of hard substrate epifauna and fish, marine mammals and seascape perception) such BACI design cannot be implemented and appropriate adaptations were made (Brabant et al., 2009).

The foundations (i.e. hard substrates) of wind turbines are considered as a type of artificial reefs and are supposed to influence the marine environment (Fig. 2). In this PhD thesis the presence, behaviour and ecology of the hard substrate associated fish is addressed. This is done within the regular monitoring measurements but mainly based on targeted more in-depth research (process monitoring) to investigate and unravel the mechanisms driving species-specific fish behaviour.

Windmill artificial reefs

Natural reefs are defined as hard compact substrata on solid and soft bottoms, which arise from the seafloor in the sublittoral and littoral zone. They are either biogenic concretions or of geogenic origin and may support a zonation of benthic communities of algae and animal species as well as concretions and corallogenic concretions (definition from the European Council Directive on the Conservation of Natural Habitats and Wild Fauna and Flora; FFH Directive 92/43/EEC, 1992). Natural reefs fulfil important ecological functions and provide distinct ecosystem goods and services: they provide spawning sites, refuge and protection options, attachment sites and food for several trophic levels (Bellwood et al., 2004; Krone, 2012; Pike and Lindquist, 1994). Besides natural reefs, artificial reefs (ARs) exist as well and have been introduced to the marine environment for many decades.



Figure 2. The marine environment around an offshore wind turbine with. All organisms represented are supposed to be influenced by the artificial hard substrate. Species list is not limiting and organisms are not drawn to scale. With the permission of C-Power and Dredging International. 1. Harbour porpoise, 2. common mussel, 3. plumose anemone, 4. mackerel, 5. common starfish, 6. horse mackerel, 7. *Pisidia longicornis*, 8. *Jassa herdmani*, 9. pouting, 10. Atlantic cod, 11. edible crab, 12. swimming crab, 13. plaice, 14. lemon sole, 15. common sole, 16. dab, 17. brill, 18. *Lanice conchilega*, 19. *Spiophanes bombyx*, 20. *Urothoe brevicornis*.

Benthos: Fauna and flora living on, in, or near the sea bed.

Epibenthos: benthic organisms living on the sea bed. They may also be attached to vertical structures in the water column (e.g. shipwrecks, wind turbines) from the low-water mark up to the bottom. Some examples are crabs, sea anemones, amphipods and starfish.

Macrobenthos: benthic organisms that are greater than 1 mm in size and live buried or burrowing in the sediment, often in the oxygenated top layer. Some examples are polychaetes, bivalves, echinoderms and larger crustaceans. Synonym: macro-endobenthos

Demersal fish: fish living on or near the bottom of the sea or lake. Demersal fish can be divided in two types: benthic fish and benthopelagic fish.

Benthic fish: fish living on the bottom of the sea or lake. They have negative buoyancy. Some examples are dab, plaice and common sole. Synonym: groundfish

Benthopelagic fish: fish living in close association with the bottom of the sea or lake, but do not rest on the bottom. They have neutral buoyancy. Some examples are pouting, Atlantic cod and sea bass

Pelagic fish: fish living in the sea or ocean at mid-water or surface levels. Some examples are mackerel and horse mackerel

Textbox 1. Faunal groups present in the marine environment (relevant for this study), based upon Lawrence (1995).

These ARs are generally defined as “objects of natural or human origin deployed deliberately on the seafloor to mimic characteristics of natural reefs and to influence physical, biological and socio-economic processes related to living marine resources” (Jensen, 1998; Seaman, 2000). However, man-made structures with another primary goal (so-called secondary artificial reefs; e.g. wind turbines, breakwaters, oil platforms) and all material unintentionally lost at sea (e.g. cargo, shipwrecks) should be considered ARs too (Bohnsack and Sutherland, 1985; Zintzen, 2007). They are not placed at sea with the intention to create hard substrates, but they do serve the same functions. The broader definition of an AR then becomes “submerged objects of natural or human origin deployed on the seabed, mimicking some characteristics of natural reefs and influencing physical, biological and socio-economic processes related to living marine resources” (Seaman, 2000; Zintzen, 2007).

Artificial reefs have been deployed by artisanal fishermen for millennia, to create fishing grounds close to their villages. These ARs were constructed with natural materials (clustered rocks, pyramids of sticks and poles, palm leaves) available in the coastal

environment (Polovina, 1991; Seaman, 2008). In the past 20 to 30 years ARs have become a popular tool and their use, modification and expansion have increased rapidly all over the world. No longer only available natural products are used, but also (man-made) materials of opportunity (even toxic materials) are deployed; ranging from car wrecks, over tyres to fabricated structures of concrete, steel or plastic (Jensen et al., 2000; Polovina, 1991; Seaman, 2008).

ARs serve different goals; which might be important for both mankind and the ecosystem involved: 1) fisheries enhancement; 2) habitat restoration and mitigation; 3) habitat protection; 4) recreational activities; 5) scientific research (Jensen et al., 2000; Relini et al., 2007; Seaman, 2002, 2007).

Fisheries production is the oldest and most ubiquitous objective for AR deployment worldwide. ARs are known to attract and concentrate fishes and some epifaunal species (e.g. crabs, lobsters and sea urchins) (Krone and Schröder, 2011; Langhamer and Wilhelmsson, 2009; Leitao et al., 2008). As a result fishing effort and yield are favoured; with the same effort more fish can be caught (Polovina, 1991). A second goal of ARs is habitat restoration and mitigation. Worldwide natural reefs are in strong decline and deterioration through disturbance and damage by human activities (mainly through fisheries activities and pollution) is an ongoing process. Often the natural reefs are not capable to restore themselves due to various ecological dynamics (e.g. loss of sources, decline in species richness, shifts in species dominance) (Abelson, 2006; Ammar, 2009). Artificial reefs may be used as a tool to help restore disturbed habitats and mitigate adverse impacts on the ecosystem. Also as protective measure for vulnerable habitats, ARs have some potential. This protective function is mainly efficient against (illegal) trawling activities; so-called anti-trawling reefs. Successful protective reefs have for instance been installed in Spain, Italy and Portugal (Jensen et al., 2000; Relini et al., 2007). ARs may also be successful to decrease the negative impact of diving activities. The deployment of ARs nearby natural reefs may reduce the human pressure on the latter (Leeworthy et al., 2006; Polak and Shashar, 2012). Another goal of ARs is the initiation of recreational activities. SCUBA diving and recreational fishing are popular activities at ARs and both types of activity are rapidly increasing businesses (Polak and Shashar, 2012). In recent years, ARs also serve for hypothesis testing (Bortone, 2006). Several reefs have been deployed to answer specific research questions. The best known example is the 'Suwannee Artificial Reef Complex' in the Gulf of Mexico (Frazer and Lindberg, 1994), in which different interspaces between reef units were applied to investigate the functional ecology of reefs.

Besides advantages, ARs may have some disadvantages as well. The use of toxic materials to construct ARs may pollute the marine environment. Scrap tires for instance, may result in the leachage of toxic substances from the surface of the tires (Hartwell et al., 1998).

Further, ARs may act as a stepping stone for the spread of some non-indigenous epifaunal and fish species as opportunities become available for species that were previously unable to settle due to the lack of available hard substrates (Kerckhof et al., 2011). ARs may also induce some issues related to shipping. Existing navigation routes for small vessels for instance can be blocked and a detour may be necessary (Snyder and Kaiser, 2009).

An important parameter of ARs is the design. The design will have a major influence on the reef ecology and on the specific functions the AR might serve. Specific designs will attract specific species and age groups depending on their habitat requirements (Brickhill et al., 2005; Pickering and Whitmarsh, 1997). The area and volume of the reef, the reef height and profile, the complexity, the composition of the reef material and the location all have their influence on the reef carrying capacity and effectiveness (Bohnsack and Sutherland, 1985; Pickering and Whitmarsh, 1997). Reefs with many crevices and openings of different sizes allow a more diverse species community and variations in vertical relief of the reef enhance the variability in water flow, turbulence patterns, sedimentation rate and light levels, catering diverse species requirements (Brickhill et al., 2005; Pickering and Whitmarsh, 1997). As the current research will focus on windmill artificial reefs, a short overview of the possible foundation designs of these wind turbines is given.

Different types of foundations exist for offshore wind turbines, each with specific dimensions and technical qualifications (Fig. 3). Most commonly used foundation types for shallow and intermediate water depths are monopiles and tripods, but gravity based and jacket foundations are used as well (Malhotra, 2007).

- The monopile is a steel pile (with a diameter up to 6 m) that is driven into the seabed to a specific depth. On top of the pile a transition piece is added which connects the pile with the wind tower. On the seabed, normally a scour protection layer of pebbles and rocks is added to prevent erosion pits to be formed around the monopile.
- Tripods have a triangular steel frame at the base of the turbine. A jacket leg is present at each corner, connected diagonally and horizontally braced to a transition piece in the centre (Malhotra, 2007).
- Jacket foundations consist of a steel jacket with four (or sometimes more) legs. Four pin-piles (with a much smaller diameter as monopiles) are driven into the seabed and the legs of the foundation are grouted on the pre-piles (Brabant et al., 2011). Normally no scour protection is added around jacket foundations.

- The gravity based foundations are hollow concrete structures that are placed on the seabed. They are filled with sand and the weight of the structure itself forms a stable support. The gravity based foundation is surrounded by a scour protection, consisting of a filter and armour layer (Brabant and Jacques, 2010; Peire et al., 2009).

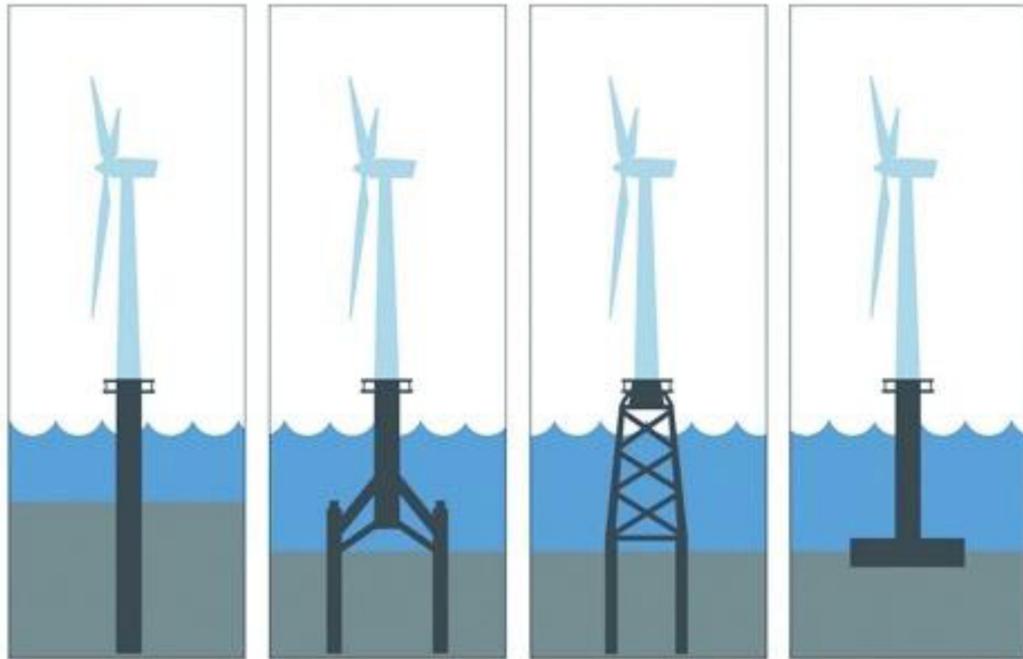


Figure 3. Foundation types for offshore wind turbines. From left to right: monopile, tripod, jacket and gravity based foundation. Adopted from Czyzewski (2012).

Artificial reefs in the Belgian part and the Southern Bight of the North Sea

Natural hard substrates in the Southern Bight of the North Sea are rarely exposed at the seabed. Most of the seafloor exists of sandy sediments with small areas with outcropping clay beds, gravel beds, pebbles and boulders (Degraer et al., 2006; Jones et al., 2004; Kerckhof and Houziaux, 2003). Many hard substrates in the Southern Bight of the North Sea however, are artificial; the majority of these ARs were unintendedly deployed (e.g. shipwrecks) or were deployed with another primary function in mind (e.g. wind turbines and oil platforms). Only few ARs have been built with the intention to create an AR. Until 2002 in the Southern Bight of the North Sea, only one reef site was known to be constructed on purpose in the Netherlands (deployed in 1992) (Jensen, 2002). This is in strong contrast with other parts of the world; in the Mediterranean Sea and the US for instance this practice is much more often executed (Jensen et al., 2000).



Figure 4. Overview of the subtidal artificial reefs in the Belgian part of the North Sea. The black stars represent wrecks, the black dots represent windmill artificial reefs. Data from the shipwrecks was obtained by the Maritime Affairs Agency (<http://www.vlaamsehydrografie.be/wrakkendatabank.htm>). The lines represent subtidal sandbanks.

To our knowledge, no more recent initiatives were set up in the Southern North Sea. However, in Belgium, an action plan (Actieplan Zeehond) has recently been proposed to deploy some reef units (reef balls) in the BPNS to promote biodiversity. The first reef balls will be constructed in summer-autumn 2013 and are intended to be placed inside a wind farm concession zone (Vande Lanotte et al., 2012).

In some cases gas and oil platforms may be converted into ARs (i.e. rigs-to-reefs) as well. This has been applied in the United States for many years. In the Gulf of Mexico, more than a 100 platforms have been partially removed and converted into reefs (Dauterive, 1999). In the North Sea, with more than a thousand gas and oil platforms present, rigs-to-reefs might be an appropriate choice for fish conservation (Jørgensen, 2012). There is one example of an experimental rig-to-reef unit (Ekofisk oil field) in Norwegian waters (Cripps

and Aabel, 2002; Jørgensen et al., 2002; Soldal et al., 2002). However, so far no rigs-to-reefs programs have been established for the Southern North Sea (Jørgensen, 2012).

Despite the few initiatives of planned ARs, thousands of unplanned ARs are present in the Southern Bight of the North Sea, mainly as shipwrecks, oil and gas platforms and wind turbine foundations. There are numerous wrecks with a known location on the seabed; most of these are shipwrecks, which is a consequence of World War I and II and the fact that this region is one of the most heavily navigated areas in the world (Maes et al., 2000; Zintzen, 2007). In the BPNS alone more than 300 wrecks are mapped (Fig. 4, data from the Maritime Affairs Agency ; <http://www.vlaamsehydrografie.be/wrakkendatabank.htm>) while the UK and the Netherlands harbour thousands of shipwrecks (Leewis and Waardenburg, 1991; Zintzen, 2007). Most probably many more wrecks, which are not yet mapped or still unknown, are present on the seafloor. Besides wrecks, there are more than a thousand oil and gas platforms present in the North Sea as well, with more than 50 % of the platforms in UK waters (Jørgensen, 2012).

Table 1. Overview of the wind farms in the Belgian part of the North Sea and their technical aspects. NI: No information available; MW: megawatt

	Turbines	Capacity (MW)	Total Capacity (MW)
<i>C-Power</i>	54	5 & 6	300
<i>Belwind</i>	110	3	330
<i>Northwind</i>	72	3	216
<i>Rentel</i>	47-78	4 - 10	289 - 550
<i>Norther</i>	100	3	300
<i>Seastar</i>	NI	NI	NI
<i>Mermaid</i>	NI	NI	NI

Recently, thousands of wind turbine foundations may be added to the list of ARs in the Southern North Sea as well. In Belgian, UK and Dutch waters offshore wind farms are planned, under construction or already operational. In the UK, 14 wind farms are operational, many of which are situated in the Southern Bight. Together these farms form an installed capacity of more than 1500 MW. In addition eight more wind farms are partially operational or under construction and 29 more are under development in the UK (den Rooijen, 2012). In the Netherlands, two wind farms are operational: 'Egmond aan Zee' and 'Prinses Amalia Windpark' (Lindeboom et al., 2011; Wiese et al., 2009). Together these farms have an installed capacity of 228 MW (96 wind turbines). In the BPNS, two wind farms are already (partially) operational: C-Power and Belwind. In the near future five more wind farms will be present (Table 1). As a result more than 400 wind turbines

will be present in the BPNS (Brabant et al., 2012; Rumes et al., 2011a; Rumes et al., 2011b). Also outside the Southern Bight, wind farm development is in progress in the North Sea. Extended wind farm programs have been set up in Denmark, Germany and the UK (den Rooijen, 2012; Krone, 2012; Leonhard et al., 2011).

Artificial reef effects

The numerous wind turbine foundations deployed on the seabed of the North Sea add a significant amount of ARs (so-called windmill artificial reefs, WARs) to the environment. These reefs will influence the surrounding soft substrate habitats substantially (ICES, 2012b). Besides, interactions within and between the hard and soft substrate habitats will occur. A thorough overview of both biotic and abiotic effects and interactions is given in Fig. 5. This overview, adopted from the ICES WKEOMB (Workshop on Effects of Offshore Wind farms on Marine Benthos (ICES, 2012b)), shows how a small alteration (i.e. the deployment of a WAR) in the environment may have substantial effects. The WAR (and on a larger scale the OWF) induces some changes in the marine environment which has an influence on local biodiversity and ecosystem functioning (Andersson et al., 2009). As a consequence, the OWFs have some environmental costs and benefits (Langhamer et al., 2009). At first, they influence the abiotic system, through habitat alteration and changes in sediment characteristics, electromagnetic fields, underwater noise and hydrodynamics. All these changes interact thereafter with other abiotic components and with the biotic system. Colonisation by epifouling organisms; community composition of soft substrate macro- and epibenthos, demersal and benthic fish; spatio-temporal distribution and migration routes of demersal fish, seabirds and marine mammals, temperature, oxygen, nutrient fluxes are all influenced and may be changing (Degraer et al., 2012; ICES, 2012b; Krone, 2012; Petersen and Malm, 2006; Reubens et al., 2013; van Deurs et al., 2012; Wilhelmsson et al., 2006). This is a very complex system and changing one link may have a snowball effect, altering the entire system. Some changes in the system may have a predictable outcome, while others will be unpredictable as they depend upon so many interactions. Besides, it is important to take the spatial scale into account. One WAR may have a negligible or very small local influence; one OWF will have some more local influence and many OWFs all over the North Sea may have an important, regional, influence on the ecosystem.

A thorough assessment of all reef effects is very hard to carry out and almost impossible. However, intensive monitoring programmes are set up by local authorities to assess the environmental impact of WARs as meticulously as possible (Degraer et al., 2012; Leonhard and Pedersen, 2006; Lindeboom et al., 2011). However, research on the impacts of OWF is still in its infancy (Langhamer, 2012) and long term and integrated

research between several member states is needed to unravel the playing reef effects and their interactions (Degraer et al., 2012; Degraer et al., in prep).

In this PhD thesis we will focus on benthopelagic fish species at WARs and the reef effects/interactions involved. ARs are known to attract and concentrate fishes and a review of the available literature confirmed that WARs often harbour high local densities of several benthopelagic fish species (Andersson et al., 2009; Leonhard et al., 2011; van Deurs et al., 2012; Winter et al., 2010) as well. However, whether the fishes are merely attracted or also production takes place is still subject to debate. This issue is known as the ‘attraction-production debate’.

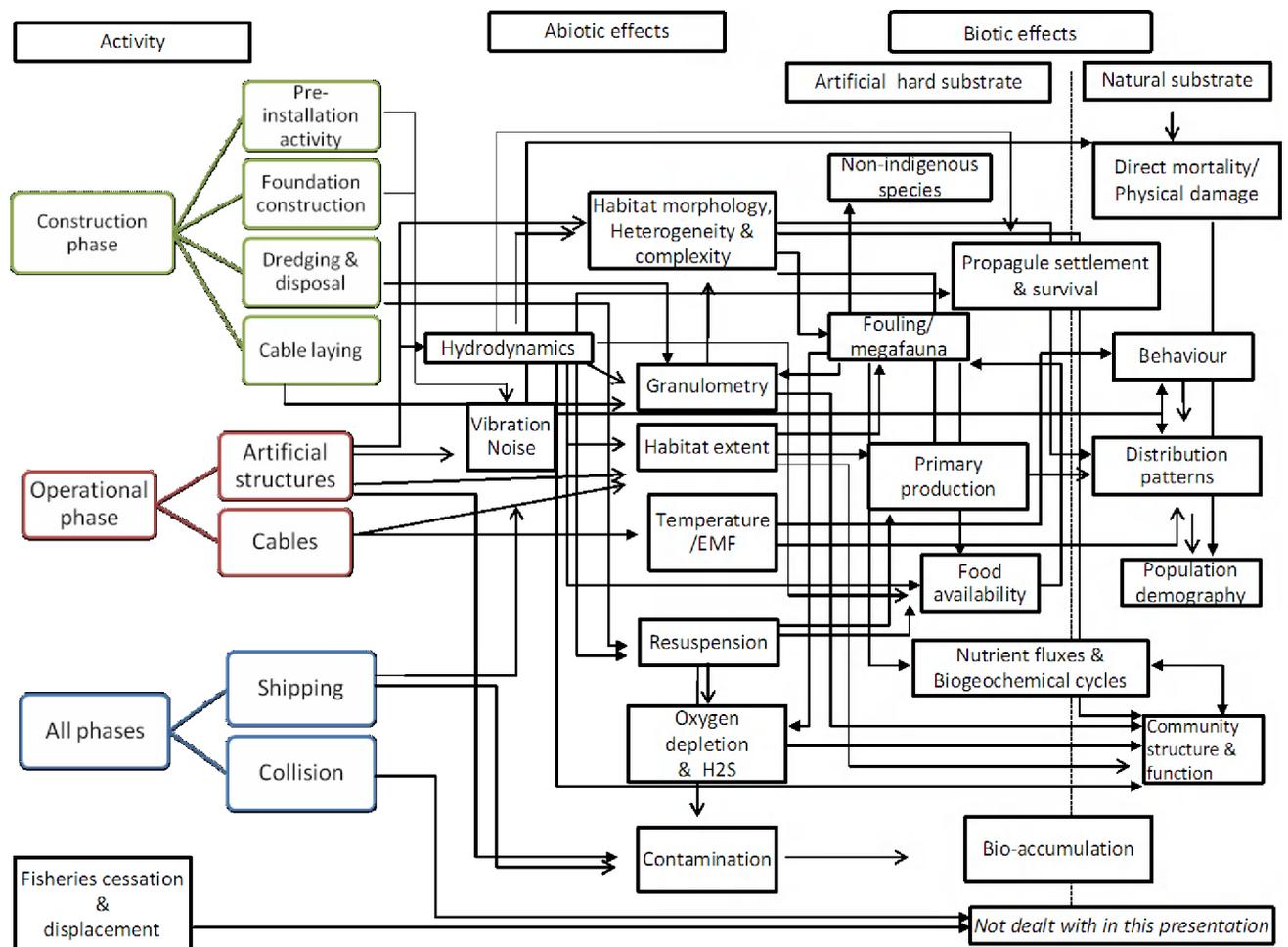


Figure 5. Overview chart of offshore wind power reef effects. Adopted from the ICES Workshop on Effects of Offshore Wind farms on Marine Benthos (WKEOMB) (ICES, 2012b)

Attraction-production debate

The complex attraction-production issue was first raised during the Third International Artificial Reef Conference in 1983 (Lindberg, 1997). By that time, ARs had become a popular tool to concentrate fishes, resulting in enhanced catches in both commercial and recreational fisheries (Bohnsack and Sutherland, 1985; Polovina, 1989, 1991). It was assumed that most reef fish stocks were limited by available habitat (Bohnsack, 1989). Increasing the amount of suitable habitat (i.e. construction of AR) would enhance the natural production of the system. Worldwide a broad variety of structures had been deployed as ARs to enhance fish stocks (Brickhill et al., 2005). However, during this conference the question was first raised whether the ARs actually do result in higher fish production or simply aggregate them at one location (Lindberg, 1997); the attraction-production debate was launched. The outcome of this issue surely has important consequences on the population dynamics of the fishes (Grossman et al., 1997) and on potential management decisions and implementations.

The *attraction hypothesis* suggests that fish move from the surrounding environment towards the reef. They aggregate at the reef, but there is no net increase in the local population. The fish are solely concentrated into a smaller area. The second hypothesis, the *production hypothesis*, assumes that the carrying capacity of the environment increases as a result of the new habitat. More fish are able to settle, survive, grow and contribute to the local population, resulting in net production (in terms of biomass and/or abundance) (Brickhill et al., 2005; Lindberg, 1997; Pickering and Whitmarsh, 1997). However, we are convinced that the attraction-production issue as it is stated in the literature is an oversimplification of reality. In the continuum of attraction and production an essential part has been neglected so far, namely *ecological traps*. In suddenly altered ecosystems ecological traps may arise. When an organism is attracted to, and settles preferably in a habitat with suboptimal conditions relative to other available habitats it is caught in a so-called ecological trap (Robertson and Hutto, 2006). Habitat choices are a consequence of natural selection and are based upon a number of ecological cues which indicate the quality status of a habitat (Schlaepfer et al., 2002). An ecological trap may occur, when changes in the environment act to uncouple the cues used to assess habitat quality from the true quality of the environment (Robertson and Hutto, 2006).

It is important to add the ecological trap to the issue as negative ecological consequences for fish may arise from the initial attraction. In the attraction-production issue, the condition of the initial fish stock present might either improve or remain as it was; while in the attraction-ecological trap-production issue (as presented here) the situation may also deteriorate.

As proposed by Hixon and Beets (1993) local abundances of fishes are determined by the relative magnitudes of recruitment by larvae, colonization and emigration by juveniles and adults, predation and competition. In addition, fishes will allocate their surplus energy from resources to growth and reproduction (Roff, 1983). So, in a simplified model; over time, fish will grow, reproduce and some mortality (both natural and fisheries induced mortality) will occur as well. For simplicity, immigration and emigration (Carr and Hixon, 1997) were left out of the model.

If we apply this simplified model to the attraction-ecological trap-production issue three theoretical outcomes are expected (Fig. 6). In the case of attraction fish' growth, reproduction and mortality in the system observed will be comparable to the reference situation. The carrying capacity of the system does not change. However, spatial dispersion of the fish changes, with aggregation in some places and reduced number in others. If an ecological trap occurs, growth is reduced and/or survival rate is lower compared to the reference situation. Although better alternative habitats are available, the suboptimal habitat is preferably chosen, resulting in reduced carrying capacity of the system. In the case of production, fish have an enhanced growth, a higher survival rate or some combination of both compared to the reference situation, resulting in an increased carrying capacity of the system.

It is important to ask the correct questions to unravel this attraction-ecological trap-production issue (Lindberg, 1997). It would be wrong to ask "Does attraction, an ecological trap or production occurs for species X?". First of all the issue is not a simple 'trichotomy' but rather a continuum in which species-specific life history behaviours determine the outcome of the issue (Bohnsack, 1989; Brickhill et al., 2005). In many cases attraction, ecological traps and production are not mutually exclusive and may interact with one another. Secondly, it is important to link the question to the correct reference condition. On what scale should the comparison be made and between which systems? Thirdly, it would be wrong to make generalizations concerning the issue. The outcome of attraction, ecological trap or production is not only influenced by species-specific life history traits as mentioned before, but also by the reef characteristics. The design, the temporal and spatial scale of deployment, location and abundance of reef units all influence fish behaviour (Bohnsack et al., 1994; Carr and Hixon, 1997; Pickering and Whitmarsh, 1997).

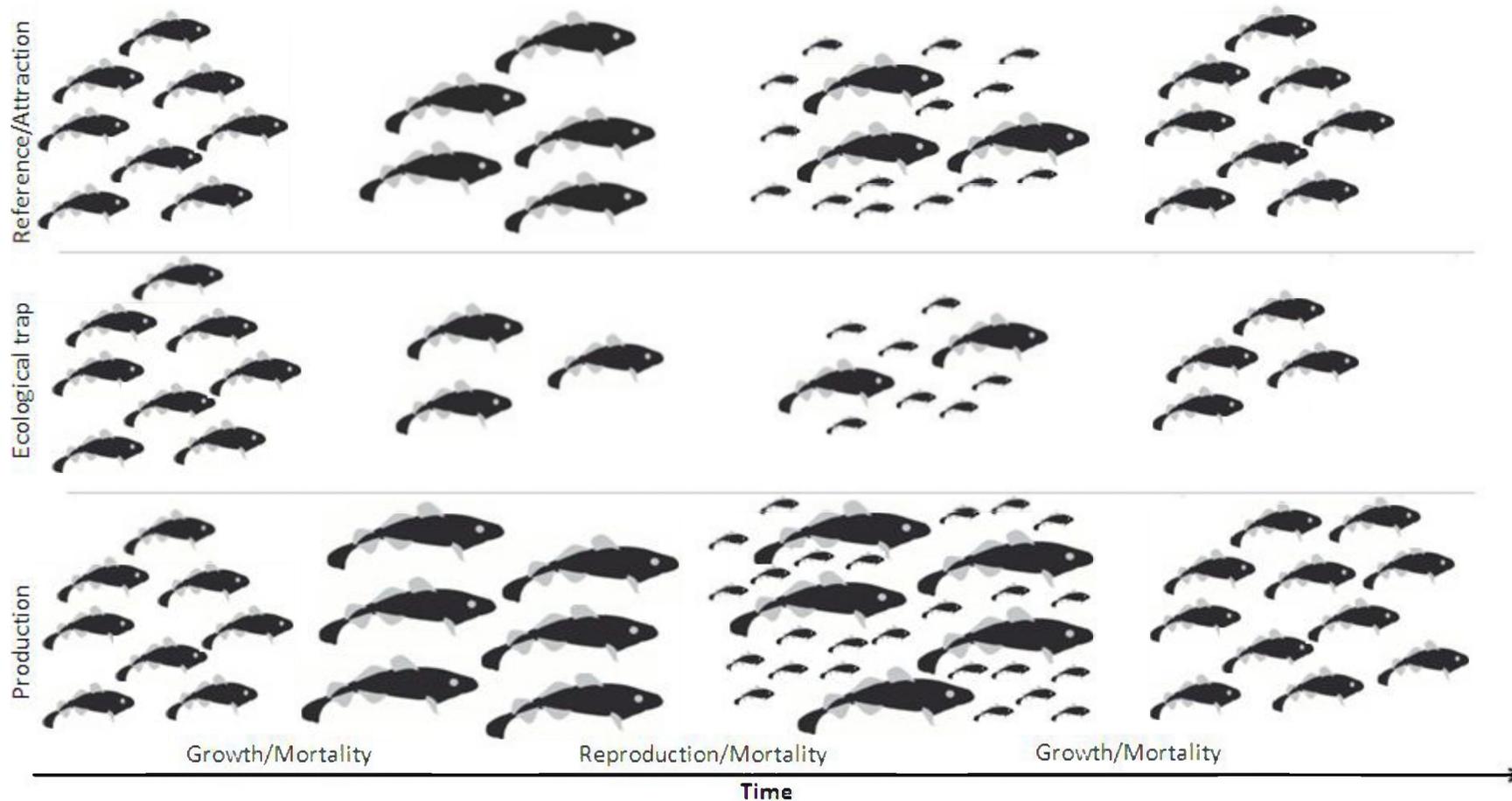


Figure 6. Conceptual representation of the 'Attraction-Ecological trap-Production Issue'

In a reference situation (upper panel) fish grow and reproduce and mortality (both natural and fisheries induced) occurs. If attraction takes place (upper panel), the outcome matches the reference situation, but spatial dispersion differs. In the case of an ecological trap (middle panel), fish have a reduced growth, a lower survival rate or a combination of both compared to the reference situation. If production occurs (lower panel), fish have an enhanced growth, a higher survival rate or a combination of both compared to the reference situation. For reasons of simplicity immigration and emigration were left out of the model.

Based on the suggestions by Lindberg (1997) and the information by Bohnsack (1989), Brickhill (2005) and Pickering and Whitmarsh (1997) we propose the following questions to unravel the attraction-ecological trap-production issue:

- 1) Does attraction of fish towards the ARs occur?
- 2) If there is attraction of fish, is it age group specific?
- 3) Which mechanisms or processes influence fish production in the ecosystem investigated? Are any of these mechanisms/processes affected by the ARs?
- 4) What is the species-specific behavioural ecology of fish in this ecosystem?
- 5) If there is production of fish, is it sufficient to offset associated fishing mortality?

These questions (see also Fig. 7) are not easily answered and the mechanisms playing are not always directly observed. The observations should be performed on an appropriate temporal and spatial scale and with many different tools in order to obtain data and information on several life history characteristics of the fish. The information obtained under questions 3 and 4 should be integrated to understand the mechanisms and behavioural cues playing and to determine the outcome of the attraction-ecological trap-production issue. It is important as well to pose question 2. Fishes can be 'attracted' to ARs at two different life stages: as (post)larvae and as older age groups (Wilson et al., 2001). The larvae arrive at the AR through direct settlement after a pelagic phase, while the older age groups arrive through active migration towards the AR. From the latter, some age groups may be attracted while others are not. Question 5 is highly significant for conservation and management decisions. If the production is not able to offset the fishing mortality, overfishing may occur (Grossman et al., 1997; Rose and Kulka, 1999). The aggregation of fish may increase the access to previously unexploited and exploited stock segments with possible deleterious effects through the increased catch rates.

Artificial reef effects on benthopelagic fishes

Artificial reefs might influence fish production through several mechanisms (Bohnsack 1989): 1) providing additional food and increasing the feeding efficiency, 2) providing shelter from currents and predators, 3) providing recruitment habitat for settling individuals that would otherwise not be able to settle; and providing suitable habitat for immigrating individuals, 4) causing stress (e.g. noise emission by operational wind turbine, increased predation pressure) (Bohnsack, 1989; Bull and Kendall Jr, 1994; Fabi et al., 2006; Leitao et al., 2007; Randall, 1963). All these mechanisms influence growth, migration, survival and/or reproductive capacity of fishes; thus having indirectly an influence on the reef carrying capacity. In Fig. 8 a schematic overview of the most important reef effects influencing fish production at WARs is given. This figure is a

deduction from Fig. 5, only indicating the most important mechanisms and processes involved.

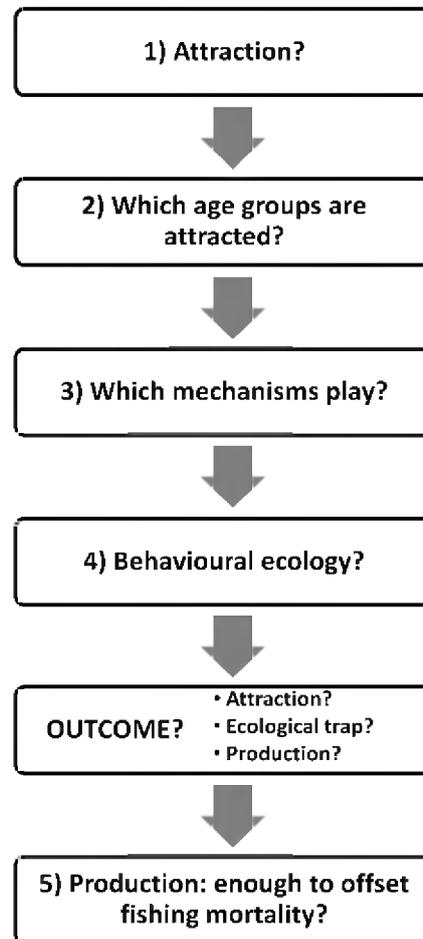


Figure 7. Schematic overview of the holistic approach to unravel the attraction-ecological trap-production issue. If questions 1 to 4 are solved the outcome of the issue is identified and eventually question 5 can be tackled.

The colonization of ARs by fish is a rapid process. Few months after installation a high number of species may already be present at the AR. Fish have in some occasions already been observed a few hours after installation (Molles Jr, 1978). An initial rapid increase in species and diversity will be followed by a stabilization of the fish assemblage structure in the subsequent months (Fabi et al., 2002; Leitao et al., 2008) or years (Relini et al., 2007). The fastest colonizers belong to the resident fish category; subsequently the transient and occasional reef fishes colonize the reef (Bayle-Sempere et al., 1994; Leitao et al., 2008). The time to reach an equilibrium community structure may take several years and the final species assemblage may depend upon environmental variables, the design and the material used (Bohnsack and Sutherland, 1985; Relini et al., 2007). Seasonal patterns in presence may be observed, certainly in temperate waters as many species migrate towards deeper water when temperature drops (Fabi and Fiorentini,

1994; Fabi et al., 2002; Leitao et al., 2008). Seasonal presences may also be related to life-history characteristics. Some fishes (e.g. Atlantic cod) are known to make extensive migrations yearly from feeding to spawning grounds (Turner et al., 2002). During the summer feeding season they may occur at ARs while during the winter they migrate towards spawning areas.

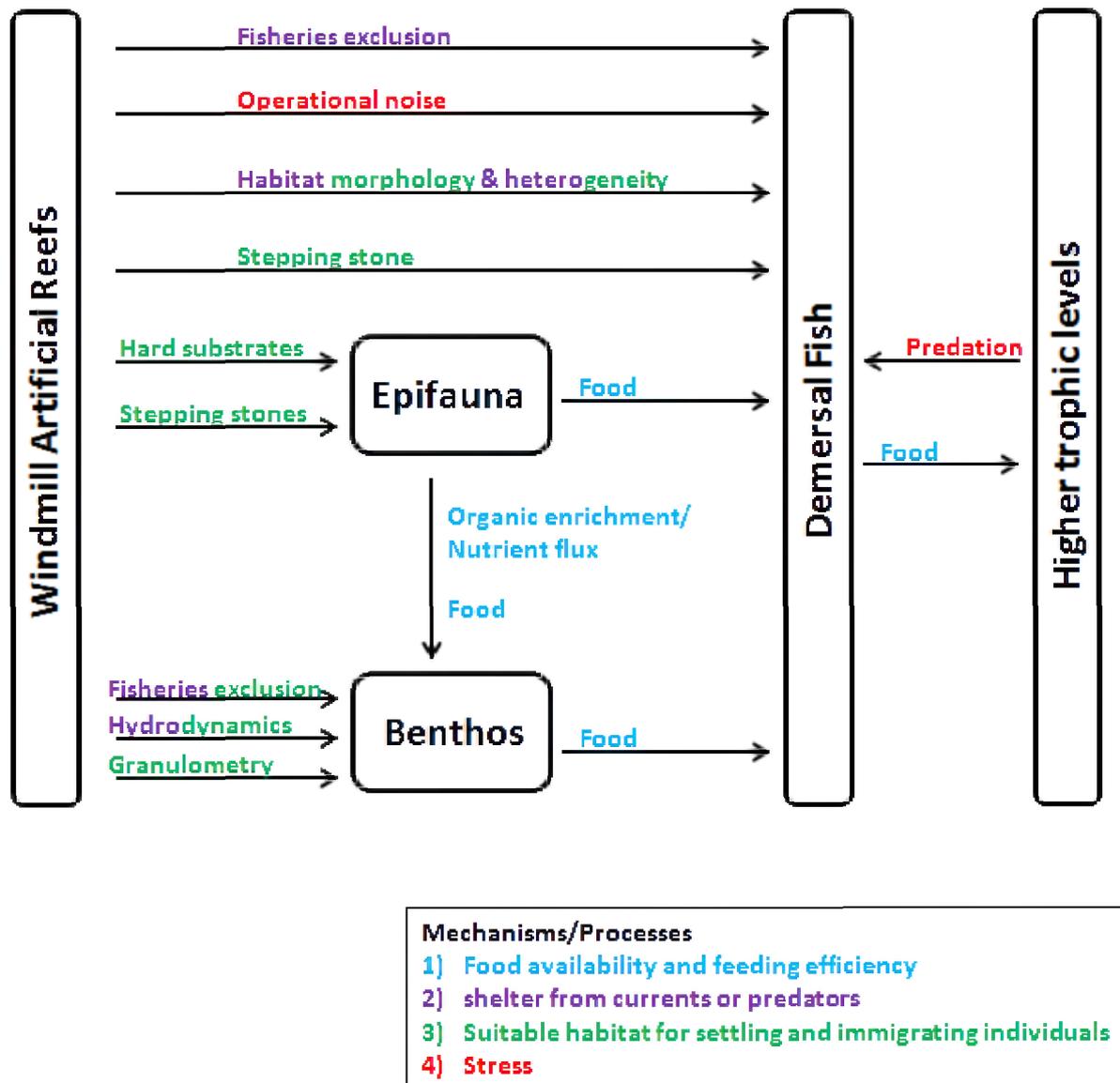


Figure 8. Schematic overview of the most important reef effects influencing fish production at windmill artificial reefs. Each mechanism/process type is indicated by the corresponding colour.

Many studies indicated that fish species may be aggregating at ARs for food. The ARs harbour a wide variety and high abundances of epifaunal organisms (Kerckhof et al., 2010a; Krone et al., 2013; Zintzen et al., 2006), both sessile and motile, which might serve as primary food source for reef fishes. Stomach content studies disclosed that many reef

associated fish species forage on organisms growing on the reef. However, some species are less dependent of the reef and feed on prey species from the surrounding soft substrates or one a mixture of soft and hard bottom associated prey (Fabi et al., 2006; Leitao et al., 2007; Lindquist et al., 1994; Relini et al., 2002).

Another important variable, besides food availability, influencing fish behaviour is the presence of shelter at ARs. Shelter will affect settlement, early survival rates and post-recruitment interactions (Hixon and Beets, 1989). It is assumed that shelter possibilities are mainly important as protection against predator attacks. Gotceitas et al. (1995) disclosed that juvenile Atlantic cod preferred the habitat type providing the best cover when predators were present. If no predators were around, no preference for a specific habitat type was observed. Also in the field specific predator avoidance behaviour related to substrate type was observed for Atlantic cod (Gregory and Anderson, 1997). Besides, prevailing currents may change due to the presence of ARs and as a result may influence the aggregation behaviour of fish. Due to the physical structure of the reef, currents deflect at the reef and a zone with higher turbulence is created. Before and after the reef a zone with low current velocity arises, while besides the reef, current velocity increases (Ecolas NV, 2006). If currents are too strong, the reef may provide protection against it. On the other hand, the currents may attract fishes, providing them with food (Jessee et al., 1985).

Marine reserves are promoted worldwide as a conservation tool for fish stocks (Roberts et al., 2001). They have proved to efficiently protect and conserve fish stocks. Fishes have higher average values of density, biomass, organism size and diversity than outside reserves (Halpern and Warner, 2002) and marine reserves also provide a refuge zone where populations of exploited species can recover (Gell and Roberts, 2003). ARs may fulfil the same function as marine reserves and in some cases the ARs were deployed specifically to protect and conserve valuable habitats and/or vulnerable fish populations (Jensen et al., 2000; Relini et al., 2007). In many offshore wind farms all fisheries and shipping activities (excluding operational activities) are prohibited (Verhaeghe et al., 2011). This safety zone, preventing collision and entanglement of fishing gear, around the wind farms can be seen as a special kind of marine reserves. In these areas, fish have a higher survival chance as a result of the absence of fishing pressure (Langhamer, 2012).

Further, ARs may act as a stepping stone for the spread of epifauna and thus also for some non-indigenous epifaunal species (Carlton and Geller, 1991; Petersen and Malm, 2006). The Southern Bight of the North Sea is dominated by soft substrates and with the introduction of new artificial hard substrates, opportunities become available for species that were unable to settle before (Kerckhof et al., 2011). Also for some non-indigenous fish species (range-expanding species) AR may form a stepping stone. In view of the warming of the oceans, some fish species (e.g. Labridae) can expand their distribution

range northward. Most Labridae however, are dependent on hard substrates (Muus et al., 1999). Although the water temperature allows a northward expansion, available hard substrates are essential to allow this expansion. With the deployment of new ARs, from wind turbines for instance, this might be the case.

The reef behavioural ecology of fishes will be influenced by several factors. The life-history reef dependency differs between species. Some species are reef obligate, while other more opportunistic species will use reefs as well as other habitats (Bohnsack, 1989). Within species, age-specific behavioural preferences may be present as well. Different age groups may occupy different habitats. After the pelagic phase, the juveniles from pouting for instance settle in estuaries where they stay a couple of months. At the end of their first year, they move to offshore areas (Hamerlynck and Hostens, 1993). Reef availability (both natural and artificial) may influence behaviour too. If a population is limited by reef availability, adding suitable habitat should result in increased abundances. However, populations may also be recruitment limited. Larval survival, dispersal or settlement survival may limit the adult populations (Bohnsack, 1989). In this case, adding more suitable habitat in the environment will not result in enhanced abundances. As last, fish exploitation pressure should be taken into account as well. Fisheries significantly limit adult (and even juvenile) population levels (Hutchings and Reynolds, 2004), certainly for long living species. Commercial fish which are reef dependent may even be more vulnerable to this limitation as they concentrate over hard substrates. This aggregation may increase the access to previously unexploited and exploited stock segments with possible deleterious effects through the increased catch rates (Lindberg et al., 2006; Polovina, 1991). As a result the behavioural ecology (i.e. ecological trades) of the species of interest may be a first indication of potential attraction or production.

Study Area

The research in this PhD thesis has been performed in the wind farm of C-Power. This wind farm is situated at the Thorntonbank, a natural sand bank in the BPNS. The BPNS is a small part of the North Sea (only 3600 km²), it is a shallow sea with an average depth of 20 m and maximum depth of 46 m (Kerckhof and Houziaux, 2003). The seafloor is characterised by sandbanks and gullies (i.e. altering deep and shallow areas). The Belgian marine waters are influenced by water masses from the Atlantic Ocean (through the English Channel). This is clear, high saline and nutrient low water. This is in high contrast with the influx of fresh water through discharges of rivers (IJzer and Schelde) in the coastal area. This water is very turbid, low saline and nutrient rich (Fettweis and Van den Eynde, 2003; Kerckhof and Houziaux, 2003). The seawater temperature varies annually between 4 and 18 °C (recorded at the Westhinder) (Fig. 9).

The wind farm of C-Power is built at the eastside of the BPNS 27 km offshore, close to the border with the Netherlands (coordinates WGS 84: 51°33'N – 2°56'E). The construction works started in 2008 and the wind farm should be fully operational by the end of 2013. In phase one (the pilot phase; period May 2007 – May 2009) six turbines were installed using gravity based foundations. In phase two and three (period Feb 2011 – end 2013), 48 more turbines were installed on jacket foundations (Brabant et al., 2012). Water depth varies between 18 and 24 m and the total surface area of the farm is 18 km².

All samples for this thesis were taken at gravity based foundations (from phase I). These foundations have a diameter of 15 metres at the seabed, at a depth of about 22.5 m at mean low water spring (MLWS). The gravity based foundations are surrounded by a scour protection layer that consists of two coats: a filter (pebble of 10 mm up to 88 mm) overtopped by an armour layer that consists of a protective stone mattress with quarried rocks (250 mm up to 750 mm)(Peire et al., 2009). Dimensions of the scour protection layer differ between the wind turbines. At turbine D5 (where most samples were taken), the armour layer has a radius of 18 m (Fig. 10). The total surface area of the hard substrates (turbine foundation and scour protection together) is approximately 2043 m² (Peire et al., 2009). The surrounding soft sediment is composed of medium sand (mean median grain size 374 µm, SE 27 µm)(Reubens et al., 2009).

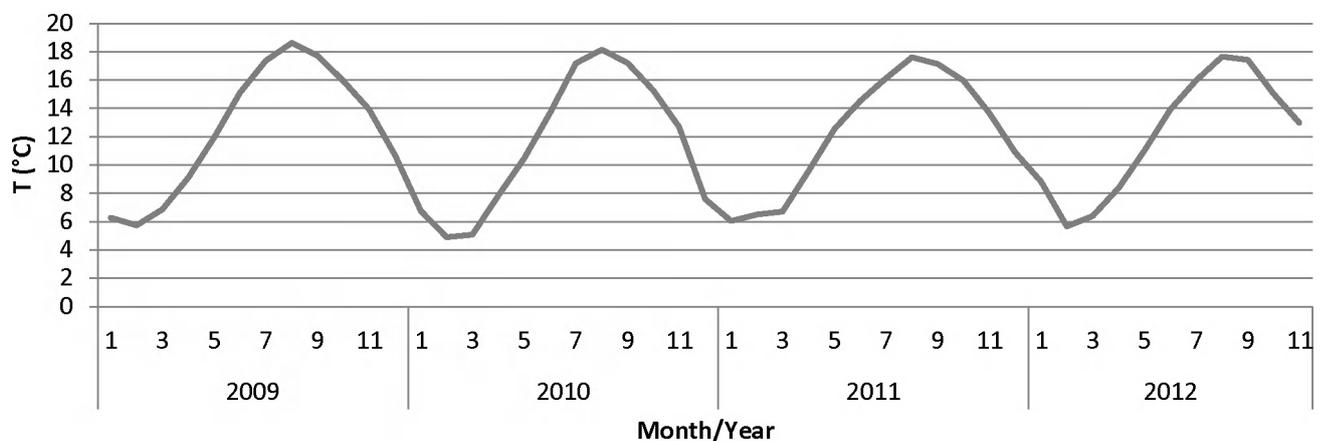


Figure 9. Mean monthly sea surface temperature (°C) over the period 2009 – 2012. Temperatures were recorded at the Westhinder. Data obtained by Vliz.

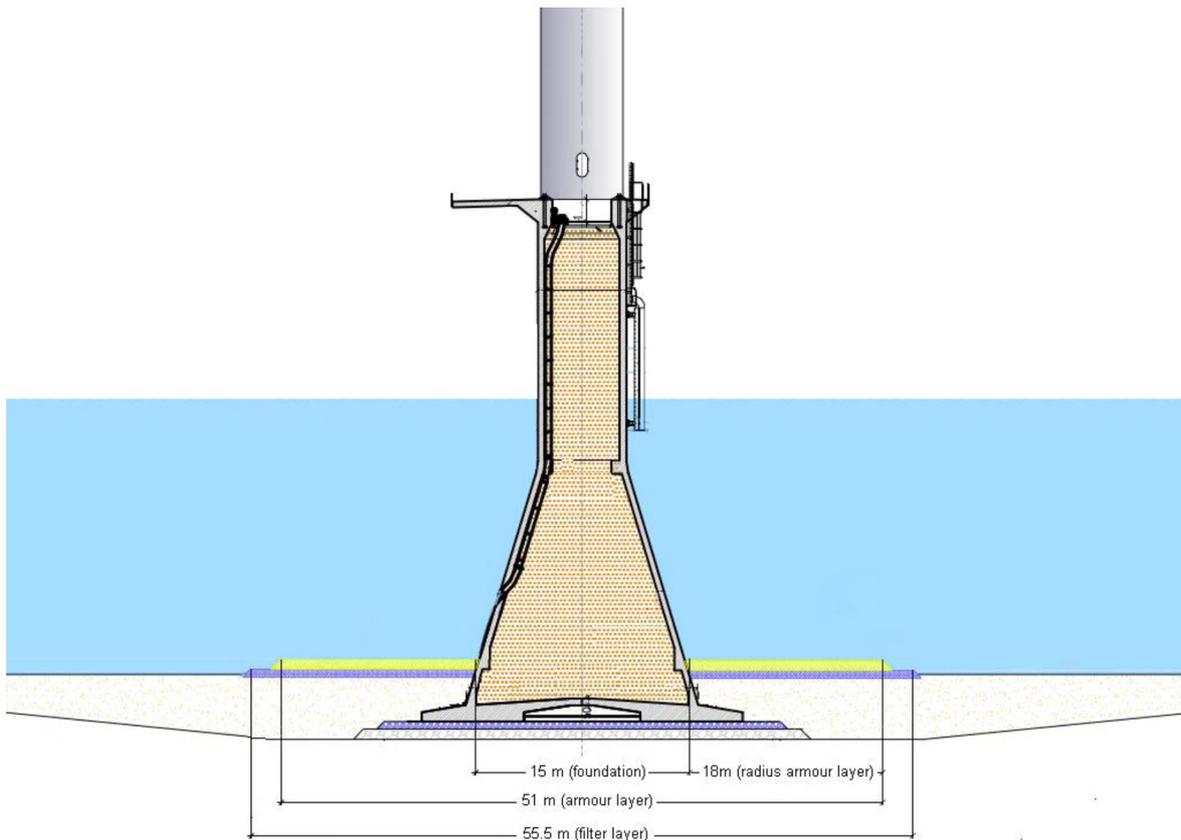


Figure 10. Dimensions of a gravity based foundation. Dimensions are based on wind turbine D5 of the C-Power wind farm.

Study species Atlantic cod and pouting

The information in this section is based upon Cohen et al.(1990), Froese and Pauly (2013) and Merayo (1996a; 1994) or mentioned otherwise.

In this study, we will focus on Atlantic cod (*Gadus morhua* Linnaeus, 1758) and pouting (*Trisopterus luscus* Linnaeus, 1758), two fish species (with high commercial value and commercial potential respectively) expected to aggregate at the WARs (see Chapter 2). Atlantic cod and pouting belong to the class of the Actinopterygii, Order Gadiformes, Family Gadidae. The Gadidae family harbours 53 species.

Atlantic cod is a benthopelagic fish species that occurs in the North Atlantic Ocean (Fig. 11). It may reach a length of 200 cm (total length, TL) and is distributed from the North American coast, over the east and west coast of Greenland, around Iceland to the coasts of North-western Europe (from the Bay of Biscay to the Barents Sea) and the Baltic. This species tolerates a wide range of salinity and temperature; from nearly fresh water to full oceanic water and from freezing temperatures up to 20°C respectively. It is widely distributed in a variety of habitats, from shoreline waters (they appear even in river mouths) up to depths of over 600 m. The juveniles prefer shallow sublittoral waters with

a high habitat complexity (Gregory and Anderson, 1997) (which provide protection from predation), while adults are usually found in deeper waters. Atlantic cod is an omnivorous and often opportunistic feeder. (Post)larvae feed on plankton, juveniles mainly on crustaceans and for older fish other fish become progressively more important in the diet. They feed mainly at dusk and dawn. Migratory behaviour differs between populations. Some cod groups are relatively stationary, while others make extensive migrations. In addition, some migrating individuals show homing behaviour while others never return to the native waters (Robichaud and Rose, 2001, 2004). Annual migrations between spawning, feeding and overwintering areas may occur (Fox et al., 2008; Metcalfe, 2006). There are Atlantic cod that mature before the age of two; however, it may take up to the age of six before all cod mature. In the North Sea the spawning period is from December to May. In the Southern part spawning will take place earlier than in the Northern North Sea. Atlantic cod is a highly valued commercial species and has been exploited ever since man began to fish the North Sea. The North Sea cod stock is considered to have a reduced reproductive capacity (mainly due to the high fishing mortality) and the exploitation is unsustainable. For more than 30 years the fishing mortality has been very high; more than 60 % of the 2 to 4 years old Atlantic cod were caught annually, which is outside the safe biological limits of the fish stock. Fishing mortality declined from 2000 and is now estimated to be around 0.4, which is still above the maximum sustainable yield of 0.2. Over the last few years however, there has been a gradual improvement in the status of the stock in the North Sea. The spawning stock biomass has increased since the historical low in 2006 and is now around 70 000 tonnes. The cod fisheries in the North Sea are managed by total allowable catches and quotas and some technical measures are taken to allow the stock to recover (ICES, 2013).

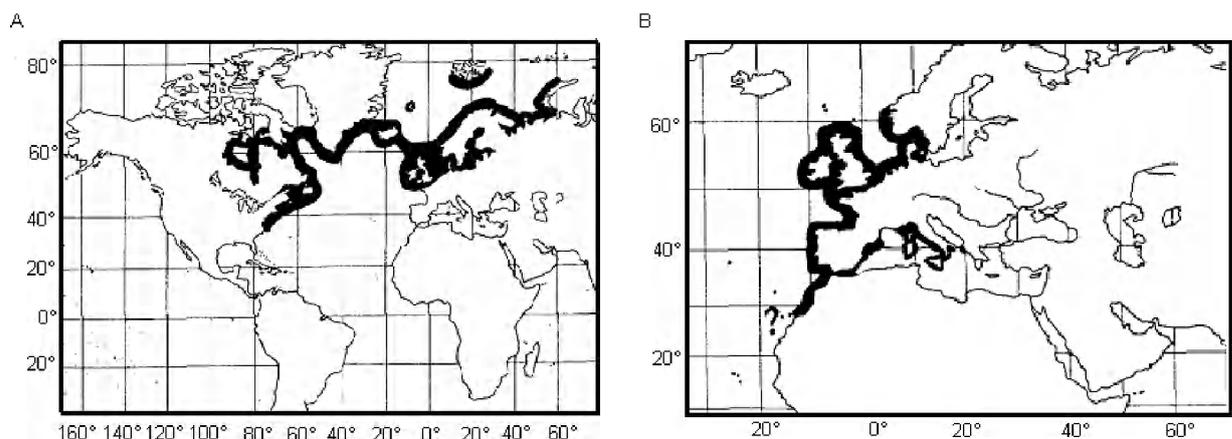


Figure 11. Distribution range of Atlantic cod (A) and pouting (B). Adopted from (Cohen et al., 1990).

Pouting is also a benthopelagic fish, reaching at most a length of 45 cm (TL). They occur at the British Isles and Skagerrak to about 25° N along the West African coast, also in the western Mediterranean (Fig. 11). Pouting occurs from inshore down to depths of 100 m, they are also found in estuaries. It lives in soft sand or rocky areas and often occurs in large schools. Pouting is omnivorous and mainly feeds on benthic crustaceans (Hamerlynck and Hostens, 1993). First maturity is reached at the end of the first year and spawning occurs from December to April in the Atlantic. Juveniles are frequently encountered in estuaries (Hamerlynck and Hostens, 1993). The species has high commercial value in southern European countries. Currently no stock assessment is performed and no information is available on the status of the fish stock.

Research objectives and outline of the PhD thesis

With the construction of offshore wind farms (OWFs), thousands of wind turbines will be present in the North Sea. The foundations form (windmill) artificial reefs (WARs) in an ecosystem that is naturally dominated by soft-bottom sediments. As a result, the OWFs induce some changes in the marine environment which may influence local biodiversity and ecosystem functioning (Andersson et al., 2009). The development of OWFs in the BPNS creates unique opportunities to investigate the impact of WARs on the ecology of benthopelagic fishes.

The objectives of the thesis are linked to the questions to resolve the attraction-ecological trap-production issue. In a stepwise approach crucial information on the community structure, behavioural ecology and reef dynamics are acquired to ultimately attain an integrated overview of the most important mechanisms and processes steering the outcome of the fish populations as observed at the WARs. The *attraction hypothesis* suggests that fish move from the surrounding environment towards the reef. They aggregate at the reef, but there is no net increase in the local population. The second hypothesis, the *production hypothesis*, assumes that the carrying capacity of the environment increases as a result of the new habitat. More fish are able to settle, survive, grow and contribute to the local population, resulting in net production. However, in suddenly altered ecosystems *ecological traps* may arise. When an organism is attracted to, and settles preferably in a habitat with suboptimal conditions relative to other available habitats it is caught in a so-called ecological trap (see also Fig. 6).

The following specific objectives were targeted in this thesis:

- (I) Unravel the fish community structure at the windmill artificial reefs. More specifically we want to know which fish species occur at the reefs and investigate seasonal dynamics in community structure.
- (II) Determine which reefs effects (e.g. food availability, shelter opportunities, habitat suitability and stress) have an impact on Atlantic cod and pouting and how these influence their behaviour at the Windmill artificial reefs.
- (III) Explore whether fishery activities can be supported inside offshore wind farms in the Belgian part of the North Sea.

The knowledge and experience obtained by this research is crucial for future management decisions for offshore wind farm projects, marine spatial planning, fisheries and potential co-user activities inside wind farm concessions (e.g. aquaculture, wave-energy concessions, nature conservation and development).

In Fig. 12 the outline of the thesis is presented, coupled to the stepwise approach for investigating the specific objectives of the thesis.

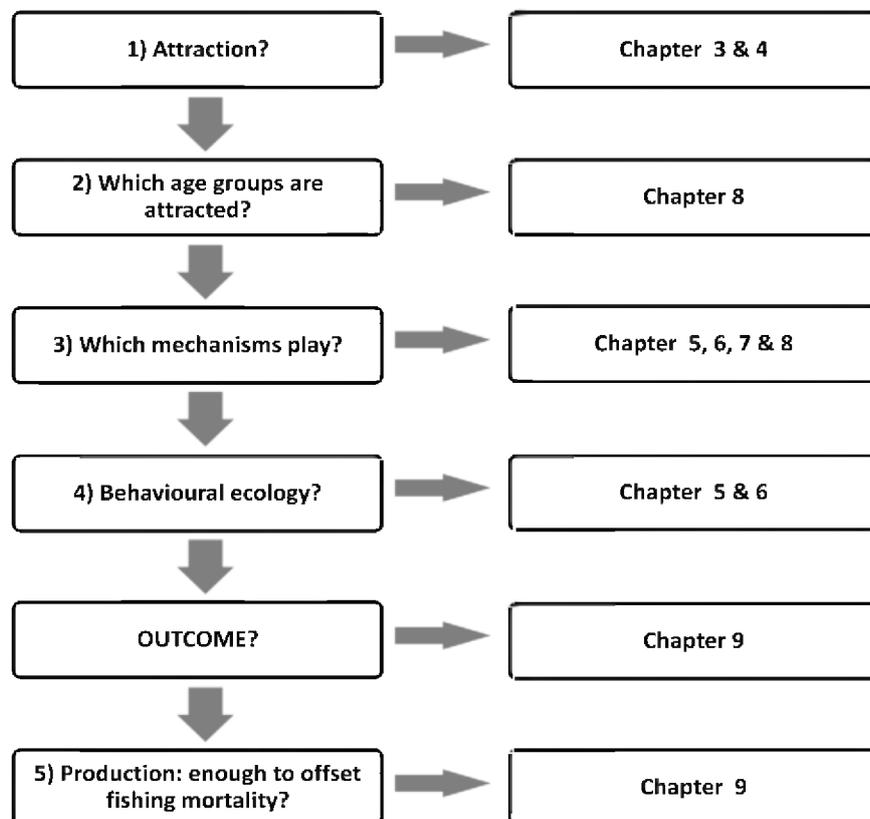


Figure 12. Overview of questions addressed in each chapter.

The thesis is a compilation of research articles (chapters 3-8 and 10) which are published, in press or under review in peer-reviewed journals. The different chapters are intended to form stand-alone units, which can be read separately. Inevitably, some overlapping information may be present in the different chapters. The chapters are organised in four different parts. Part I evaluates the community structure at the WARs. Part II focuses on attraction, while in part III the possible reef effects influencing this attraction are investigated. In part IV the acquired knowledge is integrated and discussed in relation to ecological processes, fishery activities and sea use management in offshore wind farms.

Part I. (Chapter 2) Fish community structure at windmill artificial reefs.

In chapter 2 an overview of the fish species observed at the WARs and two reference areas is given and the community structure and seasonal dynamics observed are described and briefly discussed. Two different sampling techniques are used to investigate the community: hand line fishing and visual observations with divers. The observed patterns for Atlantic cod and pouting at the WARs are discussed in more detail in the following chapters and related to the reef effects and to behavioural ecology.

Part II. Attraction towards windmill artificial reefs?

The first question to be addressed to resolve the attraction-ecological trap-production issue is whether the species of interest is attracted towards the AR? Here, we analyse catch rates of pouting (chapters 3 & 4) and Atlantic cod (chapter 4) at the WAR to investigate their potential aggregation behaviour.

Chapter 3 - Aggregation and feeding behaviour of pouting

In this study insights on the population structure of pouting at WARs are provided; count data from diver observations are used to make an estimation of pouting densities and biomass at the WAR. The aggregation behaviour is linked to food availability at the wind turbines and diet analyses were performed to broaden the knowledge on trophic relationships between pouting and resident epifaunal organisms at WARs.

Chapter 4 - Aggregation of Atlantic cod and pouting at different habitats

To determine the importance of different habitat types in the BPNS for Atlantic cod and pouting, spatio-temporal variability in catch per unit effort (CPUE) data are compared between WARs, a ship wreck and sandy bottom areas. Intensive sampling (i.e. two-weekly to monthly) was done at three locations from 2009 until 2011. Hand line fishing was performed at three locations, to allow proper sampling close to the ARs.

Part III. Reef effects influencing behavioural ecology of Atlantic cod and pouting

Once attraction towards the WARs is confirmed, it is important to ask why this aggregation takes place. In this part we focus on the potential reef effects playing and how these mechanisms and processes influence the behaviour of Atlantic cod and pouting. Different techniques were used to unravel interactions and link mechanisms to behaviour. Acoustic telemetry; stomach content, proximate and fatty acids analyses; visual observations and line fishing were combined to get more insight in movements, presence, residency and site fidelity linked to feeding ecology, predation, fisheries exclusion, energy profiling and protection.

It should be kept in mind that the *in situ* investigation of behaviour of fishes is a logistically hard task and is in some cases even impossible. Which technique is most appropriate depends upon the fish ecology and logistic possibilities (i.e. access to site, financial situation). In this study acoustic telemetry was selected to investigate the behaviour of Atlantic cod. Acoustic telemetry is a reliable technique offering valuable information on natural spatio-temporal movement behaviour.

Chapter 5 - Residency, site fidelity and habitat use of Atlantic cod

In this chapter the seasonal presence in an OWF of Atlantic cod was investigated. Therefore 22 specimens were acoustically tagged and monitored for up to one year. Besides seasonal presence, in-depth analysis of residency, site fidelity and habitat use during summer and autumn were determined as well.

Chapter 6 - Diurnal activity and movement patterns of Atlantic cod

To reveal true added value of WARs it is important to take diel variation in potential attraction into account. In this study we integrated acoustic telemetry with stomach content analysis and catch rates to quantify diel activity and evaluate diel feeding patterns of Atlantic cod at the WAR. The outcome in activity pattern was linked to possible mechanisms (i.e. food availability and shelter against currents or predators) influencing this behaviour.

Chapter 7 - Energy profiling of demersal fish

Not only should the trophic interactions be studied to determine the potential food supply at ARs; but also whether the habitat can support the necessary energy needed to maintain a specific population. Therefore energy profiling and trophic markers were applied to investigate the feeding ecology of Atlantic cod and pouting. Proximate composition in combination with fatty acid analyses of the fishes and some of their prey were compared.

Chapter 8 – Productivity at offshore wind farms

In this chapter we investigated whether the wind farms in the BPNS act as ecological traps for pouting and Atlantic cod. Ecological traps may arise when an organism is attracted to, and settles preferably in a habitat with suboptimal conditions relative to other available habitats. Therefore length, condition and diet composition of fishes present at the WARs were compared to local and regional sandy areas. Data from the period 2009-2012 were evaluated and fish were analysed per age group.

Part IV. General discussion: an overview of four years of research

In this last part all the knowledge acquired during the PhD study is integrated and discussed in a broader perspective and in relation to ecological processes, fishery activities and sea use management in offshore wind farms.

Chapter 9 - Synthesis of four years of research – where are we now?

An overview of all results obtained during the study is given. Data was gathered from 2009 until 2012 and information on length-frequency distribution, diet, community structure and movements of both species were combined to gain insights on the behavioural ecology of and to unravel whether production occurs at WARs for Atlantic cod and pouting. The outcome of the attraction-ecological trap-production issue is given and the life history of both species at the WARs is described. Further, it is discussed whether small-scale fishery activities should be allowed inside the wind farm.

Chapter 10 - Considerations, conclusions and recommendations

The last chapter, some findings are questioned in relation to methodological considerations, ecological processes and anthropogenic interactions. Finally, recommendations for future research on the ecology of benthopelagic fish species at offshore wind farms are formulated.



Part I.

Fish community structure at windmill artificial reefs



Chapter 2

Overview of the fish community structure at different habitats in the Belgian part of the North Sea

1. INTRODUCTION

In 2008 the first offshore wind turbines were built in the Belgian part of the North Sea (BPNS)(Brabant et al., 2009). The foundations of these turbines form artificial hard substrates in an environment previously dominated by a sandy seabed. These hard substrates, the so-called windmill artificial reefs (WARs) influence the ecosystem functioning and the local biodiversity. Interactions within and between the reef and the surrounding soft substrates will occur (Andersson et al., 2009; Wilhelmsson et al., 2006). One of the ecosystem components likely to be influenced is fish. Several fish species, such as pouting, Atlantic cod, sea bass, whiting and horse mackerel are frequently observed in close proximity of shipwrecks (Mallefet et al., 2008; Zintzen et al., 2006) or wind turbines (Hvidt et al., 2006; Leonhard et al., 2011).

We will investigate the benthopelagic fish community structure near WARs in relation to spatial and seasonal variability, in closer detail. To relate the community structure at the WARs to the surrounding marine environment, this habitat is compared with hard substrate (i.e. shipwrecks) and soft substrate (i.e. sandy areas) control areas. An overview of fish species and their abundance at different habitats in the BPNS and in the different seasons is given. Based on this overview two fish species will be selected, based on abundance and economic importance, for investigation. The life-history of these species in relation the WARs will than be analysed in closer detail in the following chapters.

2. MATERIAL & METHODS

The WARs investigated are located at the Thorntonbank, a natural sand bank 27 km off the Belgian coast. Each WAR exists of a concrete gravity based foundation together with the scour protection layer and has a total surface area of approximately 2043 m² (Peire et al., 2009). The surrounding soft sediment is composed of medium sand (mean median grain size 374 µm, SE 27 µm) (Reubens et al., 2009).

The hard substrate control exists of two shipwrecks, the soft substrate controls are two sandy areas (Fig. 1). Both at the hard and soft substrate controls similar environmental conditions to the wars are present at the level of current conditions, water masses, depth and grain size of surrounding soft sediment.

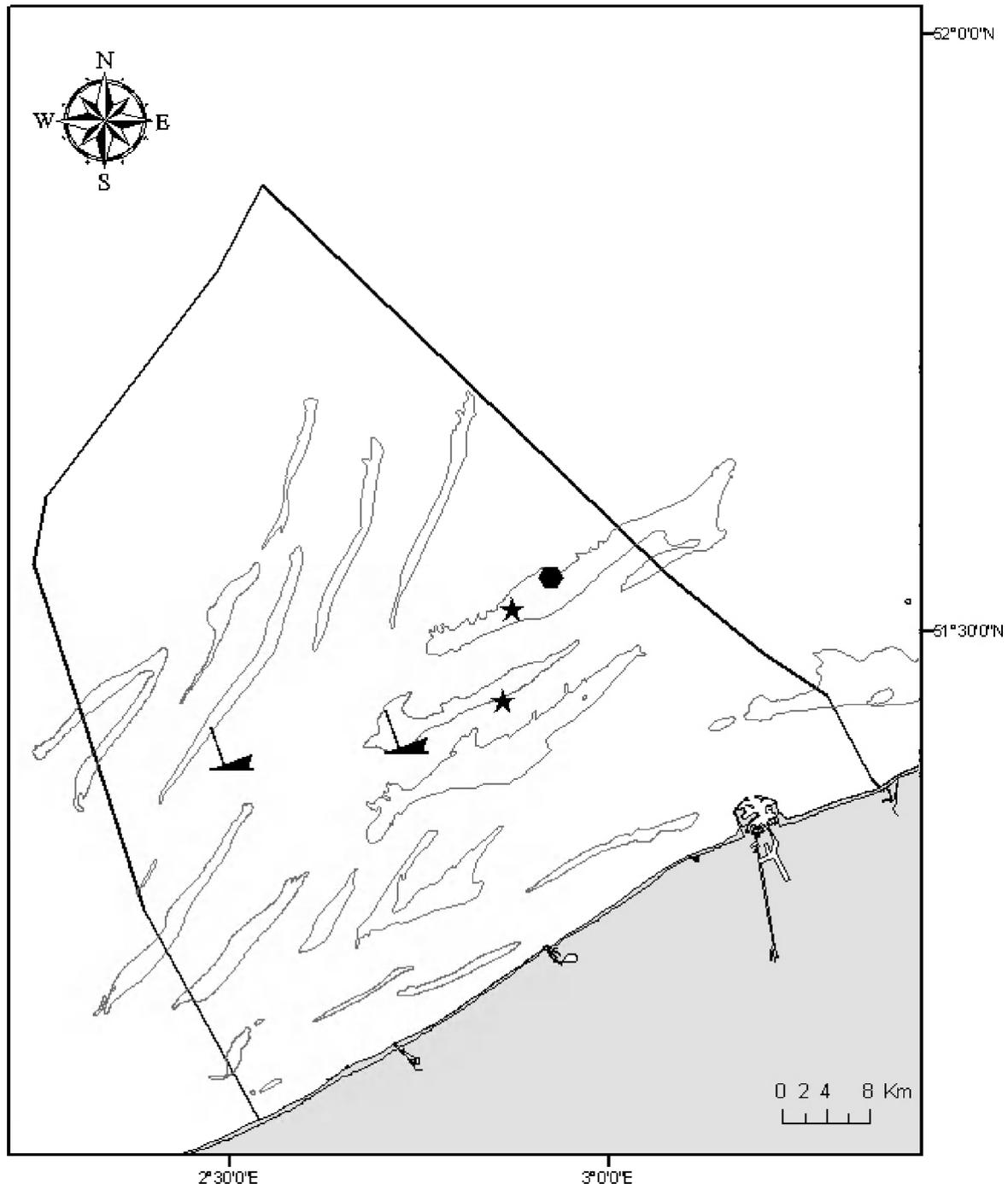


Figure 1. The Belgian part of the North Sea with indication of the sampling locations at the windmill artificial reefs (diamond), sandy areas (stars) and shipwrecks (wrecks). The lines represent subtidal sandbanks.

Two different sampling techniques were used to investigate the community structure: line fishing and visual observations. Samples were gathered from January 2009 until December 2012 on a two-weekly to monthly basis. Line fishing (hooks: Arca, size 4; bait: *Arenicola marina*) was performed at all habitats and sampling was restricted to daytime hours. Fishing time was 45 min on average and the number of fishing people ranged from 2 to 8. At the WAR angling was performed 1 to 10 metres away from a turbine (i.e. within the erosion protection layer radius) just above the bottom of the seabed, to assure that

only individuals hovering at the WAR were caught. At the shipwrecks, the research vessel drifted over the wrecks, taking into account the prevailing current and wind, and once the ship passed the wreck, it had to reposition to start over again. At the sandy bottom sites, the research vessel was anchored during fishing. Line fishing was standardized using the catch per unit effort ($CPUE = N_f/N_p * T$, with N_f the number of fish caught, N_p the number of fishermen and T the duration of fishing in hours).

At the WARs also visual observations with divers were performed. During a survey visibility was estimated and temperature and depth were recorded. All fish species encountered were listed, and abundance was estimated and behaviour observed.

All statistical analyses were performed in the Plymouth Routines in Multivariate Ecological Research (PRIMER) package, version 6.1.6 (Clarke and Gorley, 2006). All analyses are based on CPUE data.

3. RESULTS & DISCUSSION

3.1 Fish species richness, density and evenness

At the WARs both visual observations with divers and sampling through hand line fishing were performed. In total 20 fish species were observed. Line fishing revealed 12 different species, while 18 species were encountered during the dives (Table 1). Both techniques comprised species not registered with the other technique. It is interesting to note that the visual surveys revealed the presence of some soft substrate associated species (e.g. plaice, common sole and dab) as well. These species were mainly observed on the scour protection, on sandy patches between the boulders (personal observations). During the scientific dives Atlantic cod, pouting, horse mackerel and mackerel were most commonly registered at the WARs. Sea bass was the only species observed once at the WARs, while species as saithe, black seabream, goatfish and wrasse were observed few times. The other species were encountered on a more frequent basis.

From all habitats together, 24 different fish species were registered (Table 1).

For the comparison between the WARs and the control areas only information from line fishing was used as no visual surveys were performed in the control areas. All three habitats had a comparable number of species caught, but CPUE strongly differed between the habitats (Table 1). At the sandy areas typical soft substrate associated fish species were caught; e.g. lesser weever, dab, plaice, flounder and sole as well as some pelagic species, e.g. horse mackerel and mackerel. The WARs are characterized by some hard substrate associated benthopelagic species (e.g. Atlantic cod, pouting and pollack) and some pelagic species (horse mackerel and mackerel). At the wrecks, species typically caught at the WARs (e.g. Atlantic cod and pouting) and the sandy areas (e.g. dab and whiting) respectively are both present but in lower numbers (Table 1).

Table 1. Species list from different habitats. Visual census was only performed at the WARs, line fishing was performed at all three habitats; * indicates single observations (from both techniques and all habitats combined). Line fishing is indicated as catch per unit effort (mean \pm SD).

Common name	Scientific name	Visual census <i>Diving</i>	CPUE (ind fm ⁻¹ h ⁻¹)		
			<i>WAR</i>	<i>Wrecks</i>	<i>Sand</i>
Dogfish*	<i>Scyliorhinus canicula</i>				0.003 \pm 0.03
Atlantic cod	<i>Gadus morhua</i>	x	4.3 \pm 5.2	1 \pm 1.1	0.1 \pm 0.3
Pouting	<i>Trisopterus luscus</i>	x	6.6 \pm 7.6	0.5 \pm 1	0.4 \pm 1.3
Poor cod	<i>Trisopterus minutus</i>	x	0.01 \pm 0.1		
Whiting	<i>Merlangius merlangus</i>		0.009 \pm 0.1	0.09 \pm 0.3	0.7 \pm 1.4
Pollack	<i>Pollachius pollachius</i>	x	0.03 \pm 0.2	0.01 \pm 0.1	
Saithe	<i>Pollachius virens</i>	x	0.01 \pm 0.1		
Tub gurnard	<i>Chelidonichthys lucerna</i>			0.004 \pm 0.02	0.02 \pm 0.1
Bull rout	<i>Myoxocephalus scorpius</i>	x	0.08 \pm 0.3		
Sea bass	<i>Dicentrarchus labrax</i>	x		0.008 \pm 0.05	
Horse mackerel	<i>Trachurus trachurus</i>	x	1.7 \pm 3.6	0.07 \pm 0.2	0.07 \pm 0.2
Black seabream	<i>Spondylisoma cantharus</i>		0.003 \pm 0.03		
Goatfish	<i>Mullus spec.</i>	x			
Wrasse	<i>Labridae spec.</i>	x			
Lesser weever	<i>Echiichthys vipera</i>			0.02 \pm 0.1	0.1 \pm 0.3
Blenny	<i>Blenniidae spec.</i>	x			
Dragonfish	<i>Callionymus lira</i>	x	0.02 \pm 0.2		
Goby	<i>Gobiidae spec.</i>	x			
Mackerel	<i>Scomber scombrus</i>	x	0.4 \pm 1.6	0.2 \pm 0.5	0.5 \pm 1.3
Dab	<i>Limanda limanda</i>	x	0.02 \pm 0.1	0.1 \pm 0.2	1 \pm 1
European plaice	<i>Pleuronectes platessa</i>	x		0.001 \pm 0.01	0.07 \pm 0.2
European flounder	<i>Platichthys flesus</i>				0.008 \pm 0.04
Lemon sole	<i>Microstomus kitt</i>	x			
Common sole	<i>Solea solea</i>	x			

The average species richness per sampling event is very low (1.3 to 3.4 species) at all three habitats (Table 2). In winter the lowest number of species is recorded, while in summer the highest number is recorded. The number of individuals caught per sampling event varies between habitat and season. At the WARs the lowest number of individuals is caught in winter, in summer and autumn this number is much higher. At the wrecks and the sandy areas the differences observed between the seasons are less pronounced compared to the WARs. In all seasons the number of individuals collected at the WARs are (much) higher than at the wrecks and sandy areas, although the differences are less pronounced in winter and spring. Pielou's Evenness does not differ much between the seasons at the wrecks and the sandy areas. At the WARs the evenness is lowest in autumn, in this season catches are dominated by Atlantic cod and pouting. Between the habitats the evenness is comparable, except for autumns.

Table 2. Overview of average species richness, catch per unit effort (CPUE) and evenness (Pielou) per sampling event at the different habitats per season. The CPUE is calculated as the number of fish caught by one fisherman in one hour. If zero or one species was present in a sample, no evenness could be calculated. These were left out to calculate the average evenness.

	Species richness			CPUE (ind fm ⁻¹ h ⁻¹)			Evenness		
	WAR	Wreck	Sand	WAR	Wreck	Sand	WAR	Wreck	Sand
Winter	1.5	1.33	1.34	3.46	0.67	1.82	0.84	0.86	0.76
Spring	2.19	1.94	2.26	9.89	1.35	2.77	0.81	0.8	0.74
Summer	3.27	3.38	2.5	21.41	3.06	2.68	0.78	0.89	0.8
Autumn	2.44	3.0	2.49	20.06	3.63	3.43	0.62	0.76	0.84

3.2 Community structure

Figure 2 gives an overview of the community structure at the different habitats. At the WARs Atlantic cod and pouting dominate the catches in all seasons. In winter some bull rout are caught as well. In spring and summer horse mackerel is an important species in the community and mackerel contributes in summer months. At the shipwrecks Atlantic cod is the dominant species throughout the year while pouting is mainly observed in summer and autumn. In spring and summer horse mackerel and mackerel contribute to the community, while whiting contributes in autumn and winter. At the sandy areas whiting and dab are dominant in all seasons except summer. In spring and summer mackerel is an important contributor to the community. To a lesser extent horse mackerel and lesser weever contribute as well in these seasons.

If we have a look at the average CPUE per month, it is clearly visible that many species show seasonal variability in presence (Fig. 3). In general, across all three habitats, horse mackerel and mackerel are species typically present in our regions in late spring and summer, while whiting and dab are typical for autumn and winter. Atlantic cod and pouting are observed throughout the year, but in winter and early spring very low catch rates are encountered (1.3 and 0.8 ind fm⁻¹ h⁻¹ for Atlantic cod and pouting respectively). Atlantic cod abundances peak in summer (20.5 ind fm⁻¹ h⁻¹), while those of pouting peak in late autumn (20.9 ind fm⁻¹ h⁻¹). At the WARs, very high abundances of Atlantic cod and pouting are observed compared to the shipwrecks and sandy areas (Fig. 3). The dominant species from the WARs (i.e. Atlantic cod, pouting, mackerel and horse mackerel) are also observed at the wrecks, be it in lower abundances. At the sandy areas whiting and dab dominate the catches in winter and spring, while mackerel peaks in summer. Pouting reaches high CPUE in late summer and autumn.

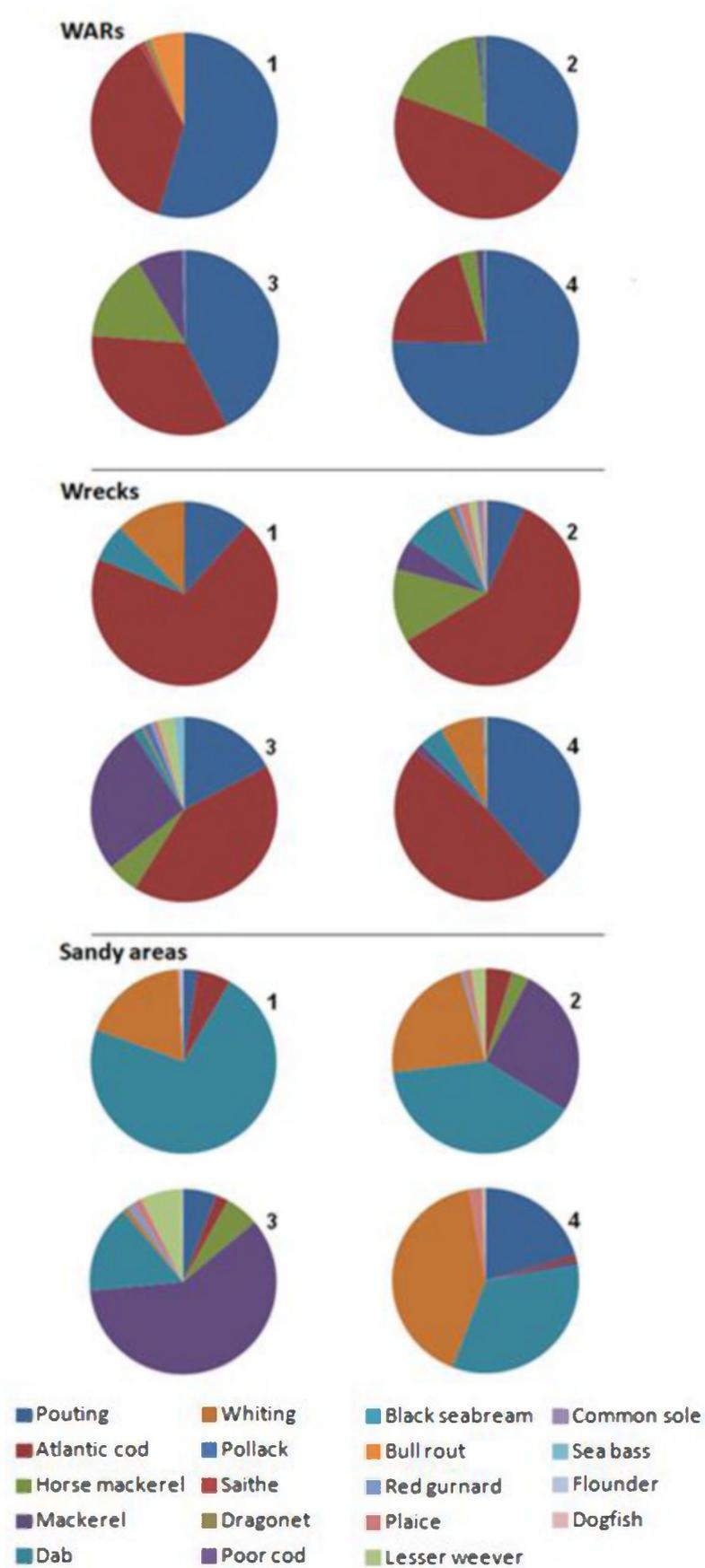


Figure 2. Fish community structure at the different habitats and between seasons. 1 winter, 2 spring, 3 summer, 4 autumn.

These observations are confirmed by multivariate analyses. Non-metric multidimensional scaling (MDS) showed the three different habitats to be clearly distinct groups, with the wrecks in-between the WARs and sandy areas in distance (Fig. 4). Within each group, the community structure in winter was most distinct from the one in summer and autumn. The analysis of similarity (ANOSIM) revealed significant differences between habitat and season (two-way crossed). The similarity percentages (SIMPER)(two-way crossed for habitat and season) show largest dissimilarities (95 %) between the WARs and the sandy areas, with Atlantic cod, pouting and dab explaining most of the differences. The WARs and the wrecks display lowest dissimilarities (81 %), with mainly Atlantic cod and pouting explaining the differences. Average dissimilarity between the shipwrecks and the sandy areas is 87 % with dab, Atlantic cod, pouting and whiting as the species explaining most of this dissimilarity. If the seasons are examined (across all habitats), it shows that average similarity within seasons increased from winter to autumn; with 22, 26, 34 and 42 % respectively. Average dissimilarity is highest between winter and summer (85 %) with mackerel, pouting, Atlantic cod and dab explaining most of the dissimilarity, while it is lowest between spring and autumn (74 %) with pouting, whiting, dab and Atlantic cod as the species explaining most of the dissimilarity.

Most research and monitoring performed to investigate the impact of offshore wind farms on the fish community structure works on the scale of the wind farm (Hille Ris Lambers and ter Hofstede, 2009; Leonhard et al., 2011; Vandendriessche et al., 2012). In the current study however, investigations are done on wind turbine scale. To our knowledge, this is the first study to investigate the changes in the environment on such a detailed scale.

Line fishing is known to be a selective fishing method and is influenced by type and size of baits, hook design, hook size, fishing strategy and fish ecology (Erzini et al., 1996; Løkkeborg and Bjordal, 1992; McClanahan and Mangi, 2004; Ralston, 1990), which may explain the low species richness observed in our study. As a result, the findings on the community structure should be interpreted with care and it has to be considered as an underestimation of the total species diversity in the area.

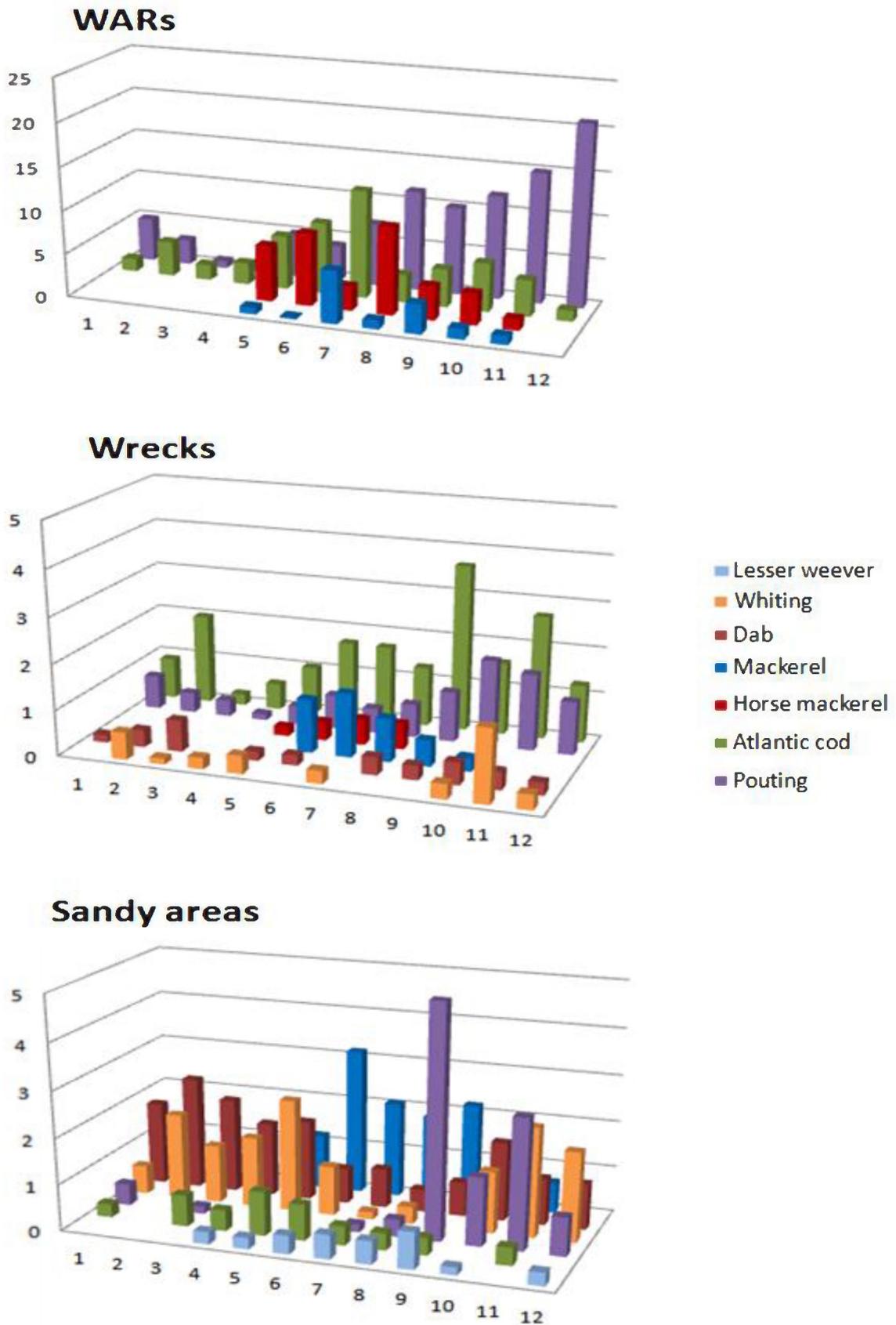


Figure 3. Average Catch per unit effort (ind fm⁻¹ h⁻¹) per month of the most important fish species at three habitat types in the Belgian part of the North Sea.

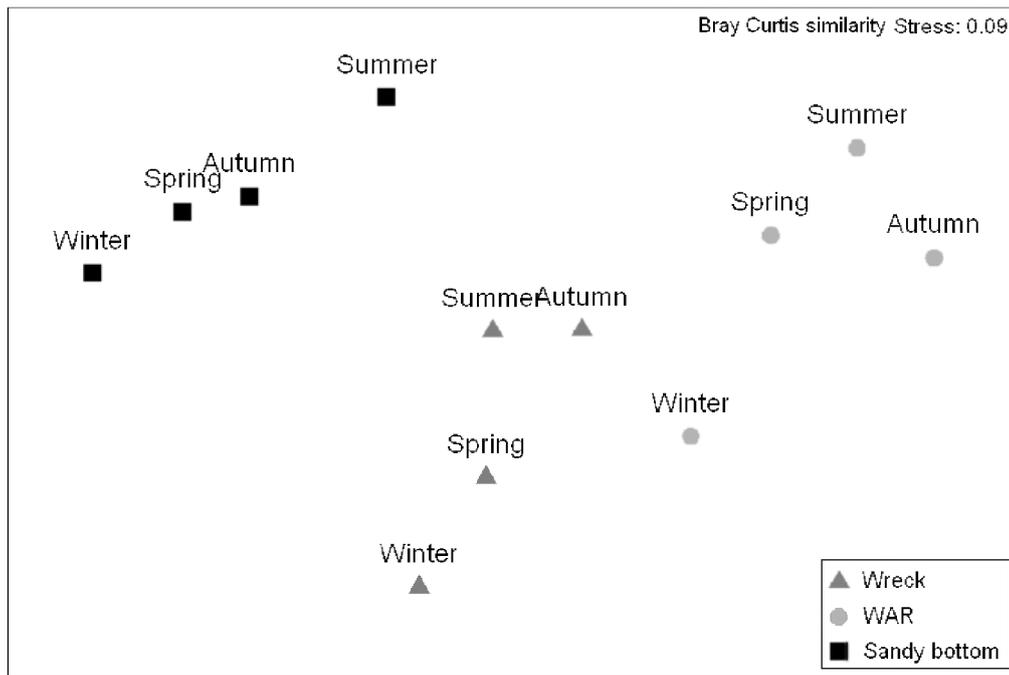


Figure 4. Non-metric multidimensional scaling showing the average across all samples of habitat*season.

We revealed the presence of several fish species near the WARs in the BPNS. The fish community structure at the WARs is significantly different from other available habitats in the BPNS and seasonal variations were observed as well. The results are in agreement with the findings from Hille Ris Lambers and ter Hofstede (2009). Atlantic cod and pouting are the most dominant species at the WARs. As these species are present in such high abundances and have a commercial value, they are the target species in this PhD study. In the following chapters we will investigate their abundances and behavioural ecology in closer detail.





Part II.
Attraction towards windmill artificial reefs?

Chapter 3

Aggregation and feeding behaviour of pouting

Adapted from:

Reubens J, 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: 223-227

ABSTRACT

A substantial expansion of offshore wind farms in the North Sea has been planned, inducing a growing interest in the effects of these artificial habitats on the marine environment. Numerous research has been done to consider the possible effects of wind farms. However, to date little research investigated actual effects on the ichthyofauna.

This study provides the first insights into the use of the artificial hard substrates by *Trisopterus luscus* (pouting) at the Thorntonbank wind farm in the Belgian part of the North Sea.

Scuba diving operated visual surveys around one wind turbine revealed a distinctly higher pouting population size and biomass (i.e. 22 000 individuals yielding a total biomass of 2700 kg) as compared to the population size present at the soft sediments surrounding the wind turbines. Stomach content analyses further demonstrated the dietary preference for prey species that lived on the turbines (i.e. *Jassa herdmani* and *Pisidia longicornis*). Yet, the present study clearly demonstrates that wind turbines build at sea may attract fish populations considerably, possibly related to the enhanced provision of resident food items on the turbines.

Keywords: artificial hard substrates; diet; ecology; pouting; *Trisopterus luscus*

1. INTRODUCTION

An enhanced demand for green energy resources has stimulated the implementation of wind turbines at sea. These wind turbines may provide a suitable habitat for hard substrate dwelling fish (Bohnsack, 1989; Bull and Kendall Jr, 1994; Fabi et al., 2006; Leitao et al., 2007) since hard substrates, e.g. shipwrecks and other artificial reefs, have been reported to attract and concentrate fishes and/or to enhance local fish stocks (Bohnsack, 1989; Leitao et al., 2008, 2009; Pickering and Whitmarsh, 1997). Several mechanisms may stimulate this behaviour, including (1) shelter against currents and predators (Bohnsack, 1989; Jessee et al., 1985), (2) additional food provision (Fabi et al., 2006; Leitao et al., 2007; Pike and Lindquist, 1994), (3) increased feeding efficiency and (4) provision of nursery and recruitment sites (Bull and Kendall Jr, 1994).

The construction of the first wind farm in the Belgian part of the North Sea (BPNS) was initiated in 2008 at the Thorntonbank, a natural sandbank 27 km offshore. At present, six gravity based foundations have been built. In the near future a total of 54 wind turbines will be constructed on this sandbank, creating an area of 0.0864 km² of artificial hard substrate and by 2020 more than 200 wind turbines will be present in the BPNS (Brabant et al., 2009). The frequent observations of several fish species such as *Trisopterus luscus* (pouting), *Gadus morhua* (cod), *Dicentrarchus labrax* (sea bass), *Scomber scombrus* (mackerel), *Trachurus trachurus* (horse mackerel) and *Pollachius pollachius* (pollack) in close proximity of ship wrecks in the BPNS (Mallefet et al., 2008; Zintzen et al., 2006) illustrates that artificial hard substrates may influence fish population distribution in the BPNS. However, (1) quantitative information on the fish community structure around the windmill artificial reef (further referred to as WAR) and (2) knowledge on the trophic relationships between fish species and resident organisms on the WAR do currently not exist for the BPNS. This is the first study that investigates the density and diet of a commercially important demersal fish, i.e. pouting, in the vicinity of a WAR in the BPNS.

2. MATERIAL & METHODS

2.1 Study site and data collection

The density and diet of pouting occurring around the foundation of one wind turbine (coordinates WGS 84: 51°32.88'N – 2°55.77'E) at the Thorntonbank was monitored in July-October 2009. The foundation has a diameter of six metres at the sea surface expanding to 14 metres at the seabed, about 25 m deep at high tide. The foundation is surrounded by a scour protection layer that consists of two coats: a filter layer, made up by pebble (10 mm up to 80 mm) which is overtopped by the armour layer that consists of a protective stone mattress with rocks (250 mm up to 750 mm). The armour layer has a diameter of 44 m (1600 m²). The surrounding soft sediment is composed of medium sand (mean median grain size 374 µm, SE 27 µm)(Reubens et al., 2009).

Nine underwater visual censuses were carried out on the scour protection by applying a variation of the stationary sampling method (Bannerot and Bohnsack, 1986) to count pouting. Observers rotated around themselves for 180°. Fish behind the divers were not counted. Each survey lasted up to 20 minutes or until no new activity was recorded for two minutes and took place four hours after high tide or two hours before high tide. Before each survey the average visibility was estimated by tape measure and used as the radius of the area observed. Observations were limited to the first meter above the seabed and within a survey all observations were made at the same position. If large schools of fish were present, abundance groups were used to count the number of individuals since this technique considerably facilitates the enumeration process and lessens the chance of error (Bortone and Kimmel, 1991). In addition, fish lengths were assessed by comparing the fishes to a ruler attached to a writing board. The surveys were unevenly distributed over the monitored period as diving was weather dependant (Table 1). As only one wind turbine was surveyed, extrapolation of the results should be considered with care.

To quantify the contribution of preys on and around the wind turbine foundation to the diet of pouting line fishing was conducted. Angling (hooks: Arca, size 4; bait: *Arenicola marina*) was performed 1 to 10 metres away from a turbine (i.e. within the erosion protection layer radius) just above the bottom of the seabed, assuring catching individuals hovering at the WAR. After the fish were measured (total length) and weighed (wet weight), stomachs were removed and preserved in an 8% formaldehyde-seawater solution. All food components in the stomachs were identified to the lowest possible taxonomic level. Dry weight (60 °C for 48 h) and ash free dry weight (500 °C for 2 h) were measured for all separate food contents in each stomach.

2.2 Data analysis

To assess the pouting population dimension on the scour protection the number of fish per square meter in the area observed was multiplied by the surface of the armour layer, which covers an area of 1600 m², since it was assumed¹ that the fish were evenly distributed across the scour protection.

Dietary composition was assessed by the occurrence (%FO) and abundance (%A) indices (Hyslop, 1980). The abundance index can be either numeric (%N) or gravimetric (%G). For the gravimetric analysis ash-free dry weight (AFDW) was used.

$$\%FO_i = (N_i/N) * 100$$

¹ Based on recent insights it is concluded that the assumptions are not correct: 1) fish are not evenly distributed over the scour protection. Visual observations revealed that fish aggregate near the sides of the wind turbine with the high currents. The sides in the lee of the current have lower densities. 2) the surface area of the armour layer differs among turbines (between 1343 and 2465 m²). 3) The wind farm has only six wind turbines with a gravity based foundation, the remaining 48 turbines are built on a jacket foundation. Preliminary results indicate that much lower fish densities are present near jacket foundations (probably due to the fact that jacket foundations have an open structure and are not surrounded by a scour protection layer). As a result, the estimated pouting population size is an overestimation of real densities.

$$%A_i = (\sum S_i / \sum S_a) * 100$$

N_i is the number of predators with prey type i in their stomach, N the total number of non-empty stomachs, S_i is the stomach content composed by prey i and S_a the total stomach content of all stomachs together (Amundsen et al., 1996). In addition, the feeding coefficient ($Q = \%N \times \%G$) (Hureau, 1970) and the index of relative importance (IRI = $(\%N + \%G) \times \%FO$) (Pinkas et al., 1971) were used to evaluate the dietary importance of each prey category.

To investigate the feeding strategy of pouting and the importance of prey items in their diet, the multivariate Principal Component Analysis (PCA) was used. Prior to analysis the numeric and gravimetric community abundance data were standardised (De Crespín de Billy et al., 2000) and a similarity matrix was constructed using the Bray-Curtis index of similarity. To investigate temporal changes in feeding behaviour, similarities in stomach content composition were assessed for each sampling period by the analysis of similarity (ANOSIM) (Clarke and Gorley, 2006). Statistical analyses were performed using the Plymouth routines in multivariate ecological research (PRIMER) package, version 6.1.6 (Clarke and Gorley, 2006). A significance level of $p < 0.05$ was used in all tests.

3. RESULTS

3.1 Pouting density assessment

Pouting was present at all surveys near the wind turbine foundation. Densities varied between 2 and 44 specimens/m² (Table 1) with an average density of 14 ± 11 individuals/m² on the scour protection yielding an average local population of 22 000 individuals near one wind turbine foundation. A large variation in densities, however, was detected both between observers and over time (Table 1). Both juveniles (< 22 cm total length) and adults were present at the WAR, since the estimated size ranged between 15 and 35 cm (with an average of 20 cm). Based on a Length - Wet weight relationship (Merayo and Villegas, 1994), the population had a biomass of 2700 kg.

Table 1. Overview of the nine visual surveys performed at one wind turbine to estimate pouting density. Each column represents a survey. Each number (individuals/m²) in the same column is assessed by one observer. Within a survey all observations were made at the same position.

Period	July	July	July	August	September	September	September	October	October
Individuals/m ²	9	9	2	7	9	44	11	11	16
	11	7	20		32	22	6		
		4							
Visibility range (m)	3	3	5	4	2	1	3	3	2

3.2 Contribution of WAR to diet of pouting

Caught fish weighed 70 g up to 345 g and lengths varied between 17.1 cm and 29.2 cm, which indicates they belonged to year class 1 to 3 (Heessen and Daan, 1996; Merayo and Villegas, 1994). Of the 72 stomachs analysed, five were empty (6.9%). The diet of pouting contained a wide variety of prey items: 41 prey types were identified, although 17 occurred only once in the stomachs analysed (Table 2). *Jassa herdmani* and *Pisidia longicornis*, both hard substrate associated prey items, occurred most in the stomachs (%FO > 35%), while *Brachyura* sp., fish scales, *Mytilus edulis*, *Liocarcinus holsatus* and *Phtisica marina* were also frequently preyed upon (%FO > 10%). Q and IRI indicated that *J. herdmani* and *P. longicornis* were the most important prey species contributing to the diet of pouting (Table 2). Numerically, *J. herdmani* (84.6%) was most important, followed by *P. longicornis* (10.3%). All other prey species represented less than one percent of the total prey density. Gravimetrically *P. longicornis* (46.8%) contributed most to the diet of pouting, followed by *J. herdmani* (28.63%) (Table 2).

PCA sufficiently illustrated the main structure in the diet composition (with the first two axes explaining respectively 43 and 18% of the total variation in gravimetric diet composition; and 66 and 15% of the total variation in numeric diet composition) (Fig. 1 and 2). In both analyses many stomachs were positioned at the edge of one of the explanatory variables, demonstrating high selectivity for a particular prey, i.e. *J. herdmani* and *P. longicornis*, which both clearly dominated the gut contents of pouting. Only few samples were positioned near the amid of the explanatory variables which indicates that these species rather foraged on a broader range of prey species and thus expressed less selectivity for a particular prey. Furthermore, despite the observed moderate overlap in gut contents for the different samples, pouting diets significantly differed over time (Anosim $p=0.001$, $R=0.254$ and $p=0.001$, $R=0.304$ for gravimetric and numeric data respectively). *J. herdmani* dominated the gut contents at all times, but *P. longicornis* was rarely preyed upon in July while this species became a dominant prey item in September.

	SPECIES	%FO	% N	% G	Q	IRI
	Hydrozoa					
H	Unidentified sp.	4.48	0.03	0.01	<0.01	0.21
	Nematoda					
N/A	Unidentified sp.	10.45	0.38	0.02	0.01	4.16
	Polychaeta					
N/A	Unidentified sp.	10.45	0.17	1.36	0.23	16.02
	Crustacea					
N/A	Unidentified sp.	4.48	0.03	0.04	<0.01	0.33
	Cirripedia					
H	Unidentified sp.	10.45	0.24	0.03	0.01	2.78
H	Balanidae sp.	2.99	0.07	<0.01	<0.01	0.22
	Mysidasea					
S	<i>Acanthomysis longicornis</i>	1.49	0.03	<0.01	<0.01	0.06
S	<i>Gastrosaccus spinifer</i>	4.48	0.17	0.02	<0.01	0.84
	Amphipoda					
B	<i>Amphilocheus neapolitanus</i>	1.49	0.07	<0.01	<0.01	0.11
B	<i>Stenothoe marina</i>	1.49	0.03	<0.01	<0.01	0.05
B	<i>Corophium</i> sp.	1.49	0.03	<0.01	<0.01	0.05
H	<i>Jassa herdmani</i>	80.60	84.59	28.63	2421.57	9124.92
H	<i>Phtisica marina</i>	11.94	0.85	0.03	0.02	10.52
S	<i>Megaluropus agilis</i>	1.49	0.03	<0.01	<0.01	0.06
	Decapoda					
N/A	Unidentified sp.	2.99	0.07	0.17	0.01	0.72
	Natantia					
N/A	Unidentified sp.	7.46	0.17	0.29	0.05	3.45
S	<i>Processa edulis crassipes</i>	1.49	0.14	0.31	0.04	0.67
S	<i>Processa modica</i>	1.49	0.03	0.10	<0.01	0.21
N/A	Crangonidae sp.	1.49	0.03	0.07	<0.01	0.16
S	<i>Crangon crangon</i>	1.49	0.03	0.05	<0.01	0.13
	Reptantia					
N/A	Unidentified sp.	2.99	0.07	0.14	0.01	0.61
B	Paguridae sp.	2.99	0.17	0.80	0.14	2.91
B	<i>Pagurus bernhardus</i>	2.99	0.10	1.40	0.14	4.48
N/A	<i>Brachyura</i> sp.	14.93	0.38	1.24	0.46	24.05
H	<i>Pisidia longicornis</i>	35.82	10.30	46.77	481.62	2044.33
H	<i>Macropodia linaresi</i>	1.49	0.03	0.01	<0.01	0.07
S	<i>Corystes cassivelaunus</i>	1.49	0.03	0.09	<0.01	0.18
B	Portunidae sp.	2.99	0.14	1.57	0.21	5.10
B	<i>Liocarcinus</i> sp.	1.49	0.03	2.08	0.07	3.16
B	<i>Liocarcinus holsatus</i>	11.94	0.48	5.24	2.50	68.29
B	<i>Carcinus maenas</i>	1.49	0.03	0.14	<0.01	0.26
	Bivalvia					
H	<i>Mytilus edulis</i>	11.94	0.78	0.02	0.01	9.55
	Bryozoa					
H	Unidentified sp.	4.48	N/A	1.37	N/A	N/A
	Echinodermata					
H	<i>Asterias rubens</i>	1.49	0.03	0.01	<0.01	0.07
N/A	Echinoidea sp.	1.49	0.03	<0.01	<0.01	0.05
	Pisces					
N/A	Unidentified sp.	4.49	0.10	2.01	0.21	9.45
S	<i>Callionymus lyra</i>	2.99	0.07	0.37	0.03	1.32
S	<i>Callionymus reticulatus</i>	1.49	N/A	1.73	N/A	N/A
	Others					
N/A	Detritus	8.96	N/A	2.62	N/A	N/A
N/A	Plant material	2.99	N/A	0.26	N/A	N/A
N/A	Fish scales	11.94	N/A	0.08	N/A	N/A

Table 2 (previous page). List of prey items. Frequency of occurrence (%FO), densities (%N), ash-free dry weight (%G), feeding coefficient (Q) and index of relative importance (IRI) of prey items present in the stomachs of pouting (*Trisopterus luscus*). N/A indicates that no quantification could be made or the information is missing. ^H Taxa living on hard substrates. ^S Taxa living on soft substrates. ^B Taxa found on both substrates. ^{N/A} Not applicable.

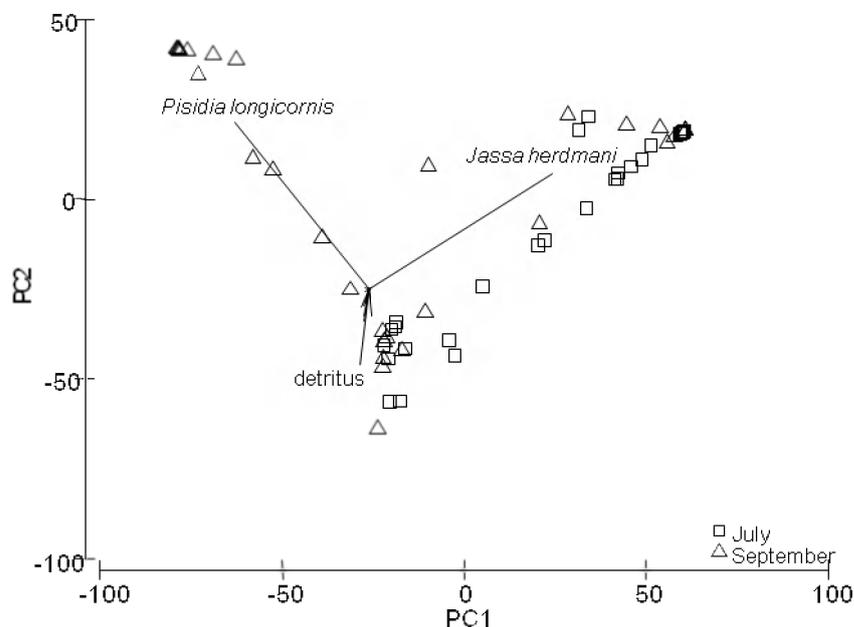


Figure 1. Gravimetric PCA based on AFDW ratio of the most important prey items. Axes 1 and 2 explain 43 % and 18 % of the total variation respectively.

4. DISCUSSION

In the BPNS pouting is frequently observed near artificial hard structures (Mallefet et al., 2008; Zintzen et al., 2006) which is consistent with the results obtained from the current research. Underwater observations indicated that a large local population of pouting (22 000 individuals, 2700 kg) was present in the vicinity of the wind turbine investigated. It can be guaranteed that the same fishes were not counted several times. During all surveys the school of pouting remained at the same position. Sometimes individuals swam against the current at the same but opposite velocity, staying in position. In other occasions they swam against the current, turned and drifted on the current, turned again and started swimming against the current once more.

Linear extrapolation¹ reveals that once the wind farm reaches its full capacity (i.e. 54 wind turbines) a biomass of 146×10^3 kg of pouting could be present. In comparison, according to FAO Fisheries and Aquaculture Information and Statistics service (2008) roughly 400 to 500×10^3 kg of pouting were landed in the Belgian harbours annually between 2000 and 2006. As only one wind turbine was surveyed, extrapolation of the results should be considered with care. Our results however do have an important signalling function as it comes to the effect of offshore wind farms on the distribution of pouting.

Interesting to note is that the population size near the WAR should be considered a minimum estimate because (1) visual census methods are known to underestimate abundant fish species (Bannerot and Bohnsack, 1986; Brock, 1982; Sale and Douglas, 1981); (2) although high densities of pouting were observed near the foundation, the estimation was restricted to the erosion protection layer, as abundances near the former are more difficult to estimate; (3) using a stationary observation method in low visibility waters induces an extra source of underestimation since individuals located at the outer edges of the visibility range are more difficult to detect and often overlooked.

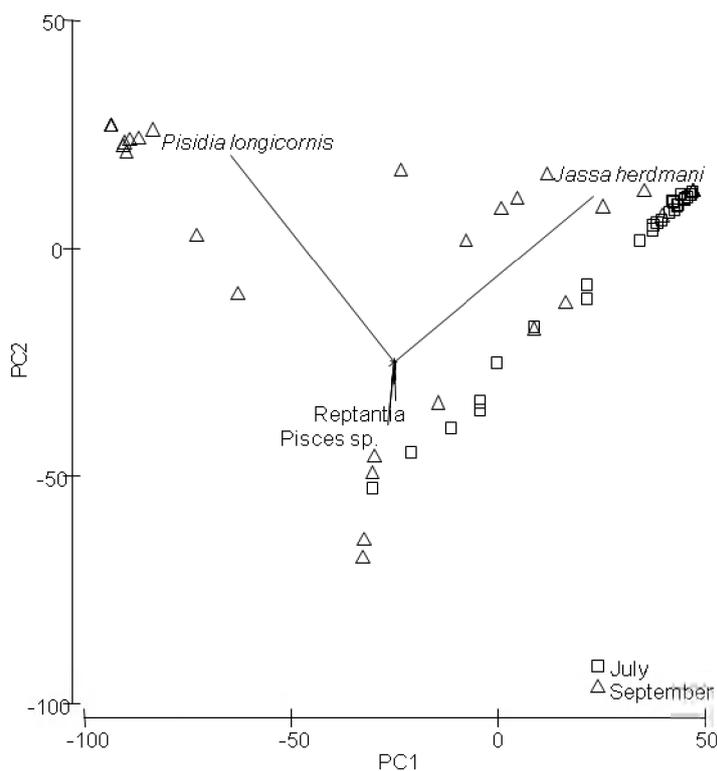


Figure 2. Numeric PCA based on density ratio of the most important prey items. Axes 1 and 2 explain 66 % and 15 % of the total variation respectively.

In comparison with pouting densities present on soft-sediments surrounding the wind turbine as retrieved from beam trawling in autumn (i.e. < 0.001 specimens.m⁻²) (Vandendriessche et al., 2009), pouting densities are highly enhanced near the WAR (i.e. 2-44 specimens.m⁻², based on visual observations). Though no information is available on beam trawl efficiency for catching Gadidae and the former comparison may be temporally biased and by the fact that pouting may respond differently to beam trawl gear versus divers, our results clearly indicate an aggregation effect of the turbines on pouting populations. Moreover, stomach content analyses clearly revealed the preference for hard substrate preys in the diet of pouting: *J. herdmani* and *P. longicornis* were the most abundant prey types. Both preferred prey species are recorded in high

densities (i.e. 100 to > 1000 individuals.sample⁻¹) at the investigated wind turbine (Kerckhof et al., 2009) and also are dominant members of the epifaunal community on the foundation of other wind farms and shipwrecks in the North Sea (Mallefet et al., 2008; Schröder et al., 2006).

Furthermore, the diet composition varied temporally which resembled the natural succession of epifauna on the WAR: *P. longicornis* only appeared in the diet from September onwards, which is the period that the species became very abundant in the epifaunal community (Kerckhof et al., 2010a).

This study provided for the first time insights on the dimension of the pouting population near a WAR in the BPNS and the importance of epifaunal food resources as one of the factors that governs the structure and spatial and temporal dynamics of the fish community. As a substantial development of offshore wind farms in the BPNS has been planned for the next coming years, the increasing number of wind turbines and subsequent biofouled scour protection could influence pouting populations. Whether these WAR increase the local pouting productivity or merely attract and concentrate the fishes is questioned and longer term investigation is needed.

ACKNOWLEDGEMENTS

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Chapter 4

Aggregation of Atlantic cod and pouting

Adapted from:

Reubens J, Braeckman U, Vanaverbeke J, Van Colen C, Degraer S, Vincx M, 2013. Aggregation at windmill artificial reefs: CPUE of Atlantic cod (*Gadus morhua*) and pouting (*Trisopterus luscus*) at different habitats in the Belgian part of the North Sea. Fisheries Research 139: 28-34

ABSTRACT

Intensive exploitation of the marine environment by mankind can alter the natural habitat of marine organisms drastically. The addition of artificial hard substrates (e.g. shipwrecks and wind turbine foundations) to soft-sediment sandy bottoms is a pervasive example of an anthropogenic habitat change. To investigate the importance of hard substrate habitats for demersal fish species, we studied the spatio-temporal variability for two commercially important species, Atlantic cod (*Gadus morhua*) and pouting (*Trisopterus luscus*), from 2009 to 2011 at three different habitats in the Belgian part of the North Sea (BPNS), i.e. windmill artificial reefs (WARs), shipwrecks and sandy bottoms. Our results showed that population densities of both species were highly enhanced at the hard substrate habitats in comparison to the sandy sediments. The highest catch-per-unit effort values for both species were recorded around the WARs, which indicated distinct aggregation around the wind turbine foundations. In addition, the observed aggregation at the hard substrates differed between seasons. Highest population densities were observed in summer and autumn, i.e. the most intensive feeding period for both fishes.

We conclude that the distribution and behaviour of Atlantic cod and pouting is affected by the presence and complexity of artificial hard substrates on the seabed.

Keywords: line fishing, aggregation, *Trisopterus luscus*, *Gadus morhua*, artificial hard substrates

1. INTRODUCTION

The marine environment is being intensively used by mankind for offshore activities and exploitation of marine resources. Man-made submerged structures for instance, are present in coastal habitats all over the world (Andersson et al., 2009; Bull and Kendall Jr, 1994; Douvere et al., 2007; Langhamer and Wilhelmsson, 2009). Adding such structures to the marine environment results in an altered habitat composition, which may influence local biodiversity and ecosystem functioning (Andersson et al., 2009; Wilhelmsson et al., 2006). In the Belgian part of the North Sea (BPNS) the seabed is mainly composed of sandy soft sediments, while natural hard substrates are rare (Mallefet et al., 2008). However, the seabed harbours numerous artificial hard substrates. Almost 300 shipwrecks are recorded in the BPNS (Termote and Termote, 2009) and numerous offshore wind turbines are currently being constructed. By 2020 more than 300 wind turbines will be present in the BPNS (Brabant et al., 2011). The significant development of artificial hard substrates induces a growing interest in the possible effects of these constructions on the surrounding marine environment. Demersal fishes are likely to be affected by the environmental changes related to the introduction of artificial hard substrates (Wilhelmsson et al., 2006). The shipwrecks and windmill artificial reefs (further referred to as WARs) provide a suitable habitat for hard substrate dwelling fish (Bohnsack, 1989; Fabi et al., 2006; Leitao et al., 2007; Reubens et al., 2011). Several fish species such as *Trisopterus luscus* (Linnaeus, 1758) (pouting), *Gadus morhua* (Linnaeus, 1758) (cod), *Dicentrarchus labrax* (Linnaeus, 1758) (sea bass), *Scomber scombrus* (Linnaeus, 1758) (mackerel) and *Trachurus trachurus* (Linnaeus, 1758) (horse mackerel) have been observed in close proximity of shipwrecks and WARs in the BPNS (Mallefet et al., 2008; Reubens et al., 2011; Zintzen et al., 2006).

Hard substrates have been reported to attract and concentrate fishes and/or to enhance local fish stocks (Bohnsack, 1989; Leitao et al., 2008, 2009; Pickering and Whitmarsh, 1997). Such aggregation behaviour can be explained by (1) increased shelter against currents and predators (Bohnsack, 1989; Jessee et al., 1985), (2) additional food provision (Fabi et al., 2006; Leitao et al., 2007; Pike and Lindquist, 1994), (3) increased feeding efficiency and (4) provision of nursery and recruitment sites (Bull and Kendall Jr, 1994). Atlantic cod and pouting are two demersal fish species with a high commercial value and a high commercial potential respectively (Alonso-Fernández et al., 2008). With regards to conservation measures, environmental impact assessments and the assessment of delivered ecosystem services in the coastal zone, increased scientific knowledge on the behaviour of both species in relation with habitat variability and complexity is paramount. The development of offshore wind farms in the BPNS creates a unique opportunity to investigate the effects of artificial hard substrates on the distribution of both Atlantic cod and pouting.

The aim of this study was to investigate the importance of three different habitats for Atlantic cod and pouting in different seasons. To achieve this, we studied the spatio-temporal variability in catch per unit effort (CPUE) and aggregation of both demersal fish species at WARs, shipwrecks and soft-bottom areas in the BPNS in the period 2009-2011.

2. MATERIAL & METHODS

2.1 Study sites

Abundances of Atlantic cod and pouting were estimated from January 2009 till December 2011 at three different habitats in the BPNS: (1) WARs, (2) shipwrecks and (3) sandy bottoms (Fig. 1). Since construction of the wind farm started only in May 2008, the WARs are considered immature artificial reefs where colonization processes (both for benthos and fish) recently started. The shipwrecks on the other hand are considered as “mature” artificial reefs, with a stabilized climax community.

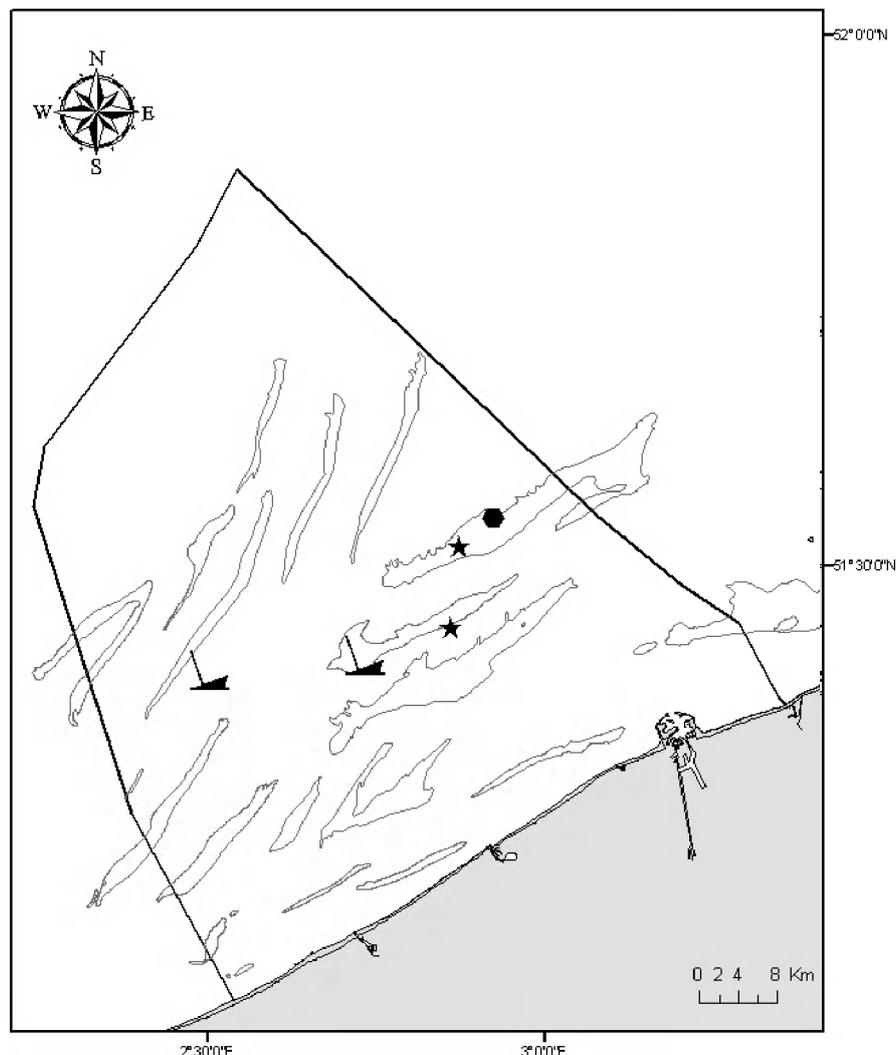


Figure 1. Map of the Belgian part of the North Sea, with indication of the sampling locations at the windmill artificial reefs (diamond), sandy areas (stars) and shipwrecks (wrecks). The lines indicate subtidal sandbanks.

The WARs are situated at the Thorntonbank, a natural sandbank 27 km offshore (coordinates WGS 84: 51°33'N – 2°56'E) at a depth of 22.5 m at mean low water spring (MLWS). The six wind turbine foundations have a diameter of 15 metres at the seabed and are surrounded by a scour protection layer (pebbles and rocks) with a total diameter of 51 m (2043 m²) (Peire et al., 2009). The surrounding soft sediment is composed of medium sand (mean median grain size 374 µm, SE 27 µm)(Reubens et al., 2009).

Two shipwrecks were monitored: the *LCT 457* (coordinates WGS 84: 51°25'N – 2°44'E) and the *Kilmore* (coordinates WGS 84: 51°23'N – 2°30'E). The *LCT 457* measures 64 m in length and 10 m wide. It is elevated between 1 m and 2.5 m from the seabed. The wreck sunk in 1944 and lies at a depth of 22 m at MLWS. It consists of iron and has little complexity. The *Kilmore* has a higher complexity, is longer (86 m) and wider (13 m) than the *LCT 457*. It is elevated up to 6 m from the seabed. The ship sunk in 1906 and the lowest part of the wreck lies at a depth of 30 m at MLWS (Termote and Termote, 2009).

The sandy bottom areas are located at the Thorntonbank (coordinates WGS 84: 51°31'N – 2°52'E) and the Goote bank (coordinates WGS 84: 51°27'N – 2°52'E) at approximately 25 m depth at MLWS. They are composed of medium sand (mean median grain size 362 µm, SE 95 and 371 µm, SE 83 respectively) (Reubens et al., 2009).

2.2 Sampling method

Line fishing (hooks: Arca, size 4; bait: *Arenicola marina*) was performed at the different sites to quantify the CPUE of Atlantic cod and pouting (Table 1). Sampling was restricted to daytime hours. Fishing time was 45 min on average and the number of fishing people ranged from 2 to 8. At the WAR angling was performed 1 to 10 metres away from a turbine (i.e. within the erosion protection layer radius) just above the bottom of the seabed, to assure that only individuals hovering at the WAR were caught. At the shipwrecks, the research vessel drifted over the wrecks and a signal was given to start and stop fishing to avoid catching fish from the surrounding soft bottom. At the sandy bottom sites, the research vessel was anchored during fishing.

2.3 Data analysis

It is well accepted that CPUE data do present useful information concerning relative abundances (Haggarty and King, 2006). Therefore, CPUE was standardized for both species as:

$$CPUE = N_f / (N_p * T)$$

with N_f the number of fish caught (ind), N_p the number of fishermen (fm) and T the duration of fishing in hours (h).

Statistical analyses were performed using the Plymouth Routines in Multivariate Ecological Research (PRIMER) package, version 6.1.6 with PERMANOVA add-on software (Anderson et

al., 2008; Clarke and Gorley, 2006). PERMANOVA makes no explicit assumptions regarding the distribution of original variables. Univariate and multivariate ANOVAs are performed with p-values obtained by permutation (Anderson et al., 2008). To investigate the spatio-temporal effects (i.e. habitats, period of the year and annual differences) on CPUE, a 3-factor design was used with fixed factors habitat (hab), month (mo) and year (ye). As the design was unbalanced at the lowest level (i.e. unequal numbers of replicate samples within each factor level of the design), it was decided to use Type I sums of squares in the analyses (Anderson et al., 2008). The lack of balance means that the various interactions and main effects cannot be estimated independently, and thus the outcome will depend on the order in which the factors are entered in the model (Anderson et al., 2008). For this study, the order used is habitat, month, year. A zero-adjusted Euclidian distance similarity matrix was used after a $\log(X+1)$ transformation of the data (Clarke et al., 2006). In case of significant factor effects, pair-wise tests were performed to investigate which groups within a factor were significantly different. In case of significant interactions, pair-wise tests within the interaction were performed (e.g. pair-wise tests of hab within hab x mo were performed to investigate in which months the habitats differed and *vice versa*). Homogeneity of multivariate dispersions was tested with PERMDISP, using distances among centroids. A significance level of $p < 0.05$ was used in all tests. Results are expressed as mean \pm standard error (SE).

Table 1. Monthly sampling periodicity at the different sites. NA: not applicable. For the sandy bottom and wreck, a frequency > 1 means either that the site was sampled several times that month or that more than one site was sampled.

Month	2009			2010			2011		
	<i>Sandy bottom</i>	<i>WARs</i>	<i>Wreck</i>	<i>Sandy bottom</i>	<i>WARs</i>	<i>Wreck</i>	<i>Sandy bottom</i>	<i>WARs</i>	<i>Wreck</i>
Jan	1	1	2	0	0	0	3	4	3
Feb	2	4	3	0	0	0	0	3	0
Mar	1	1	1	2	3	4	4	7	2
Apr	2	0	4	3	6	2	4	1	2
May	0	0	0	3	5	2	2	3	0
Jun	0	0	2	1	1	1	2	0	1
Jul	1	1	1	4	6	3	4	2	2
Aug	0	0	0	0	0	0	0	0	0
Sep	0	2	0	3	6	0	2	0	1
Oct	0	1	0	3	3	4	3	3	1
Nov	2	1	0	1	1	1	4	4	2
Dec	1	0	1	1	1	1	NA	NA	NA

3. RESULTS

3.1 Spatio-temporal variability in Atlantic cod populations

The overall mean monthly CPUE of Atlantic cod ranged between 0 and 13.6 ind h⁻¹ fm⁻¹ (Table 2). The highest mean monthly CPUE was recorded at the WARs, varying between 0.9 (March) and 13.6 ind h⁻¹ fm⁻¹ (July) (Table 2). At the shipwrecks mean monthly CPUE varied between 0.1 (December) and 3.6 (September) ind h⁻¹ fm⁻¹. Almost no Atlantic cod was caught by line fishing at the sandy bottom locations (mean monthly CPUE varied between 0 and 0.4 ind h⁻¹ fm⁻¹) (Table 2).

Significant hab x mo ($p = 0.001$) and hab x ye ($p = 0.006$) interactions in the PERMANOVA model demonstrated that differences in CPUE between habitats depended on year and month of sampling (Table 3). However, pair-wise comparisons among sampling years only revealed significant differences in CPUE between 2009 - 2010 and 2009 - 2011 ($p = 0.012$ and $p = 0.015$ respectively) at the WARs, whereas no significant differences were present between years at the other habitats. At the WARs, mean CPUE was much lower in 2009 (1.05 ± 0.4 ind h⁻¹ fm⁻¹) as compared to 2010 (6.3 ± 1.5 ind h⁻¹ fm⁻¹) and 2011 (3.9 ± 1.0 ind h⁻¹ fm⁻¹) (Fig. 2).

Table 2. CPUE (ind h⁻¹ fm⁻¹) for Atlantic cod and pouting per habitat over the period 2009-2011 (mean \pm SE). No SE value indicated the site was sampled only once during that month.

Month	Atlantic cod			Pouting		
	<i>Sandy bottom</i>	<i>WARs</i>	<i>Wreck</i>	<i>Sandy bottom</i>	<i>WARs</i>	<i>Wreck</i>
Jan	0	1.2 \pm 0.8	0.3 \pm 0.2	0	1.4 \pm 0.9	0.2 \pm 0.2
Feb	0	2.9 \pm 1.6	2.6 \pm 1.1	0	1.5 \pm 0.8	0.06 \pm 0.06
Mar	0.08 \pm 0.08	0.9 \pm 0.3	0.2 \pm 0.06	0	0.3 \pm 0.1	0
Apr	0.04 \pm 0.04	1.8 \pm 1.2	0.6 \pm 0.3	0	0.2 \pm 0.1	0.05 \pm 0.03
May	0.03 \pm 0.03	5.8 \pm 1.4	0.3 \pm 0.3	0	2.8 \pm 1.2	0.2 \pm 0.2
Jun	0.4 \pm 0.4	2.8	1.3 \pm 0.6	0	1.4	0.2 \pm 0.2
Jul	0.05 \pm 0.03	13.6 \pm 4.6	1.3 \pm 0.4	0.02 \pm 0.02	6.5 \pm 1.4	0.5 \pm 0.2
Sep	0	4.4 \pm 1.2	3.6	0	9.2 \pm 1.3	2.1
Oct	0	5.5 \pm 2.3	1.1 \pm 0.3	0.4 \pm 0.3	8.4 \pm 1.7	1.1 \pm 0.3
Nov	0.04 \pm 0.04	4.06 \pm 1.9	3.0 \pm 0.4	0.2 \pm 0.2	11.3 \pm 3.4	1.5 \pm 1.1
Dec	0	1.5	0.1 \pm 0.1	0	12.8	0.7 \pm 0.7
Overall	0.05 \pm 0.03	4.6 \pm 0.9	1.1 \pm 0.2	0.07 \pm 0.03	4.3 \pm 0.6	0.7 \pm 0.1

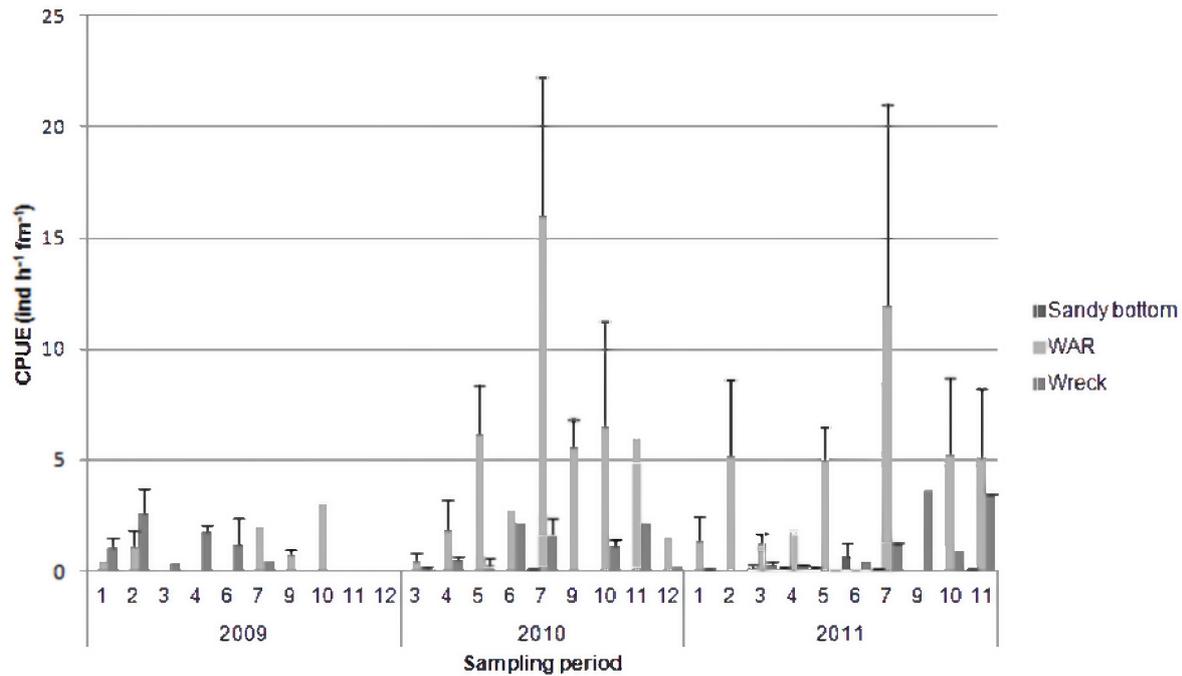


Figure 2. Mean monthly CPUE (with SE) of Atlantic cod per habitat substrate over the period 2009-2011.

A seasonal trend in CPUE was present at the WARs in 2010-2011 (Fig. 2) with significantly higher values from late spring to late autumn (with a peak in summer) compared to the winter -early spring period (December- April) (pair-wise comparisons: $p < 0.05$). At the shipwrecks a comparable, trend was visible, although CPUE was lower compared to the WARs. At the sandy bottoms, CPUE of Atlantic cod was always low and did not vary significantly over months. Pair-wise comparisons among habitats revealed that CPUE was significantly lower at the sandy bottoms as compared to the WARs from March onwards, and during summer and autumn months as compared to the shipwrecks.

Table 3. PERMANOVA results from the main test for Atlantic cod and pouting. Hab: habitat; Mo: Month; Ye: Year. P-values obtained by permutation

Factor	Atlantic cod				Pouting			
	<i>df</i>	<i>MS</i>	<i>Pseudo-F</i>	<i>P</i>	<i>df</i>	<i>MS</i>	<i>Pseudo-F</i>	<i>p</i>
Hab	2	23.54	79.79	<0.001	2	23.58	119.52	<0.001
Mo	10	1.42	4.82	<0.001	10	3.03	15.37	<0.001
Ye	2	0.60	2.02	0.13	2	0.40	2.01	0.14
Hab x Mo	20	0.82	2.77	0.001	20	1.05	5.30	<0.001
Hab x Ye	4	1.16	3.93	0.006	4	0.25	1.27	0.29
Mo x Ye	16	0.13	0.44	0.96	16	0.19	0.94	0.51
Hab x Mo x Ye	17	0.10	0.34	0.99	17	0.14	0.70	0.73

3.2 Spatio-temporal variability in pouting populations

The overall mean monthly CPUE of pouting ranged between 0 and 12.8 ind h⁻¹ fm⁻¹ (Table 2). Again, very low values (mean monthly CPUE 0 during eight months and a maximum of 0.4 ind h⁻¹ fm⁻¹) were noted at the sandy bottoms (Table 2). At the shipwrecks slightly higher values were obtained, with mean monthly CPUE varying between 0 and 2.1 ind h⁻¹ fm⁻¹. The highest mean monthly CPUE for pouting was recorded at the WARs (0.2 - 12.8 ind h⁻¹ fm⁻¹) (Table 2) with the highest CPUE registered in July 2009 (15.6 ind h⁻¹ fm⁻¹).

A seasonal trend in CPUE was present at the WARs (Fig. 3) with significantly higher values in summer and autumn (July - December) compared to winter and spring (January - June) (pair-wise comparisons: $p < 0.05$). At the shipwrecks a comparable, but less clear trend was present, except for 2009, when almost no pouting were caught at the shipwrecks. At the sandy bottom CPUE was very low during all months.

The spatial distribution of pouting also differed between months and habitats (hab x mo, $p = 0.0001$) (Table 3). Pair-wise comparisons among habitats demonstrated that CPUE was significantly higher at the WARs as compared to the shipwrecks in April, July and October; and in April, July – November when compared to the sandy bottom locations.

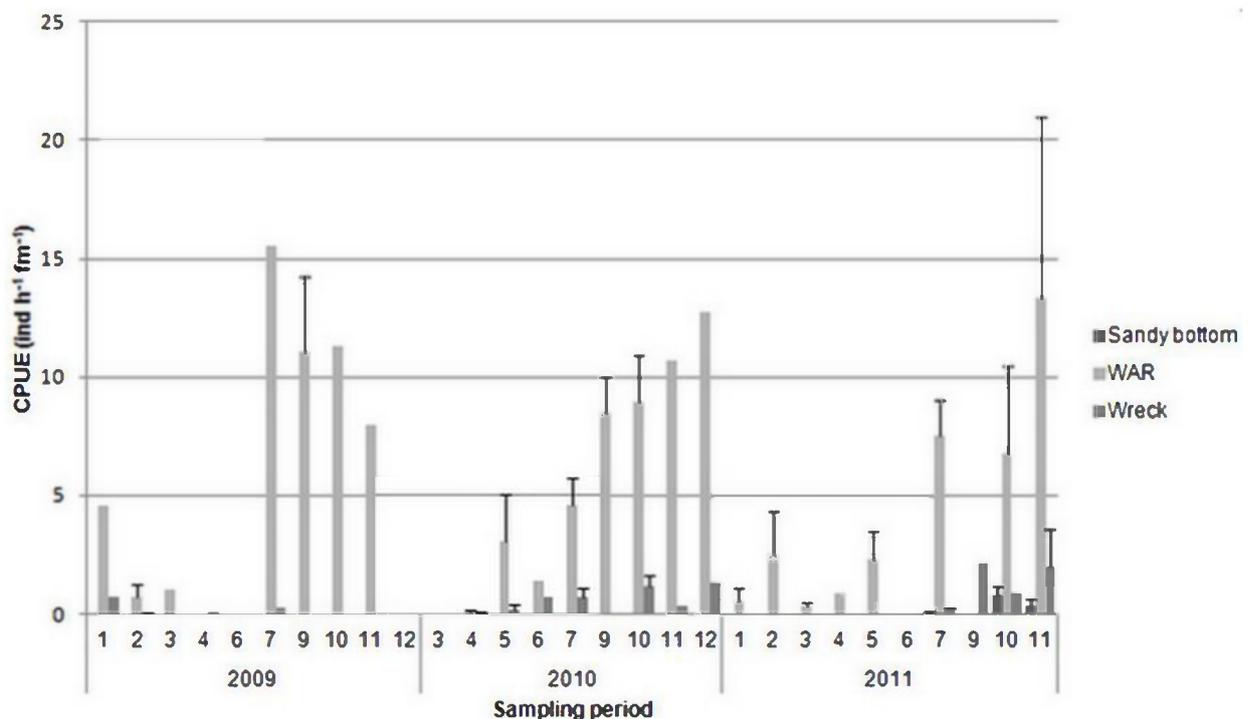


Figure 3. Mean monthly CPUE (with SE) of pouting per habitat substrate over the period 2009-2011

4. DISCUSSION

In the Belgian part of the North Sea, both Atlantic cod and pouting are frequently observed near artificial hard structures (Mallefet et al., 2008; Reubens et al., 2011; Zintzen et al., 2006). However, information concerning aggregation differences at different substrate types (i.e. sandy bottoms, shipwrecks and WARs) is lacking. Our results suggest aggregation differences, affected by seasonal aspects, for both Atlantic cod and pouting. This result is based on the analysis of a three-factor PERMANOVA model. It should be noted that, for both fish species, the assumption for homogeneity of multivariate dispersions was not fulfilled for hab within hab x mo; PERMDISP had a p-value below 0.05. This means that a significant result for one of the given factors is caused by a significant difference in location, dispersion or some combination of both between the groups (Anderson et al., 2008). Pair-wise comparison showed that CPUE at both WARs and shipwrecks differed significantly from those at the sandy sediments. However, very low within-group dispersion was present for the latter habitat (CPUE mostly near zero), which increases the differences among the centroids of the different habitats. Based on these findings we are confident that multivariate dispersions did not compromise our general conclusions. In addition, we are aware that Catch per unit effort (CPUE) not only depends upon fish densities, but also upon fish behaviour (e.g. hyperstability and habitat selection) (Linløkken and Haugen, 2006; Olin et al., 2004). In the case of hyperstability the CPUE remains high, while fish abundances decline (Harley et al., 2001; Rose and Kulka, 1999).

Seasonality

The study revealed a seasonal pattern in CPUE for both species in all habitats, which was related to life-history characteristics (i.e. reproductive behaviour of adults). Both species are known to spawn in winter and early spring (Alonso-Fernández et al., 2008; Mello and Rose, 2005a) during which they migrate to distinct spawning areas outside the study area (Franca et al., 2004; Hutchinson et al., 2001). In winter and early spring, CPUE was very low at the different habitats in the BPNS. Late spring to late autumn is the feeding and growing period for both species (Alonso-Fernández et al., 2008; Mello and Rose, 2005b), resulting in much higher CPUE, especially at the WARs and shipwrecks. Both types of artificial reefs harbour a diverse and abundant epifaunal community (Kerckhof et al., 2010b; Zintzen et al., 2008) with many potential prey species for Atlantic cod and pouting. Comparable seasonal variations in fish abundances at artificial reefs have been observed in other studies (Fabi and Fiorentini, 1994).

Habitat characteristics

A second important finding in this study is the difference in CPUE between habitats, interacting with seasonality, for both Atlantic cod and pouting. CPUE was highly enhanced

(mainly in summer and autumn) at the WARs in comparison with the sandy bottom sites. This is in accordance with results for pouting presented in Reubens et al. (2011). Although no information is available on the efficiency of catching Gadidae at different substrate types, our results clearly indicate an aggregation effect of the WARs on pouting and Atlantic cod populations. This aggregation effect was also seen at the shipwrecks, but to a lesser extent. Local factors, such as the availability of prey species (Reubens et al., 2011), predator pressure (Brickhill et al., 2005), habitat complexity and refuge possibilities (Wilhelmsson et al., 2006; Wilson et al., 2007) likely contribute to the observed differences in aggregation between the artificial hard substrates and the sandy bottom areas.

In addition it should be noted that besides reef effects also fisheries exclusion effects may explain the differences in catch rates between the habitats. The WARs are closed to fisheries activities, while in the other habitats fisheries are active. Thus, differences in fishing pressure may influence catch rates as fisheries activities lead to a decrease in fish densities (Jennings et al., 2001; McClanahan and Muthiga, 1988). However, fisheries mortality could not be estimated and was therefore not taken into account.

Several studies revealed that artificial reefs harbour higher densities of fish species compared to natural reefs or older artificial reefs. At the same time, small artificial reefs generally have higher fish densities than larger ones (Ambrose and Swarbrick, 1989; Bohnsack et al., 1994; Leitao et al., 2008). The WARs are rather small units with a high perimeter-to-area ratio. This implies that WARs can be seen as small reefs that attract fish from a larger area, relative to reef size, compared to larger reefs. This argument only applies for fish aggregation, not for production (Ambrose and Swarbrick, 1989).

Maturity of the system may influence fish assemblages. Older, more mature reefs exhibit lower fish density, diversity and biomass (Leitao et al., 2008). Fish colonization on artificial reefs is known to be associated with the epifaunal colonization (Svane and Petersen, 2001). The investigated shipwreck artificial reefs are known to have a mature and stable epifaunal community (Zintzen et al., 2008), while the epifaunal community at the relatively young WARs is still in a transitional situation (Kerckhof et al., 2010b); which surely influences the associated fish assemblages.

Time effect

A third striking result of this study are the aberrant low CPUE rates in 2009 at the WARs for Atlantic cod (Fig. 2) compared to 2010-2011. This was not the case at the other habitats. As the WARs are relatively new structures (built in 2008) constructed in an area previously dominated by soft sediments, a time effect is suggested to explain the variation in CPUE at the WARs between the different years for Atlantic cod. This corroborates other long-term investigations carried out at artificial reefs, where qualitative and quantitative increments in

fish assemblages over time were shown (Bohnsack and Sutherland, 1985; Fabi et al., 2002; Leitao et al., 2008). For pouting no such time effect was seen. From the first year onwards high CPUE rates were found at the WARs, indicating that the colonization rate of both species differed.

Currently the WARs in the BPNS are *de facto* marine protected areas. A study has however been performed to investigate the possibilities for aquaculture, blue energy development and small-scale passive fisheries inside these concession areas (Verhaeghe et al., 2011), but currently no shipping nor fishing activities are allowed inside the wind farms. Fish aggregations, as e.g. found at the WARs for both pouting and Atlantic cod, are particularly vulnerable to fishing pressure and overexploitation (Rose and Kulka, 1999). Changes in spatial distribution of a species can not only contribute to overfishing, but also to inappropriate interpretation of CPUE data. Rose and Kulka (1999) hypothesized that a concentration of fish and fishing activities can lead to an extreme hyper stability of the relation between CPUE and abundance. Consequently, CPUE will reflect local densities and therefore incorrectly assess stock abundance (Rose and Kulka, 1999). Therefore it is important to carefully monitor the aggregations of Atlantic cod and pouting in the long term. If, in the future, small-scale fisheries or aquaculture activities would be permitted within the WARs, thorough management restrictions should be implemented to mitigate possible negative effects of these activities on the fish populations.

In conclusion, the present study disclosed that the habitat type plays an important role for the distribution of Atlantic cod and pouting. Both species aggregated at the WARs and to a lesser extent also at the shipwrecks, mainly during summer and autumn, but were almost not caught through line fishing at the sandy bottoms. The aggregation is related to specific habitat characteristics (e.g. bottom type, maturity of the system and prey availability), while the seasonal patterns are related to life-history characteristics (i.e. feeding versus spawning period). A construction effect of the wind turbines was present for Atlantic cod, the first year after the wind turbines were built. This was not the case for pouting, which immediately aggregated in higher numbers around these hard substrates.

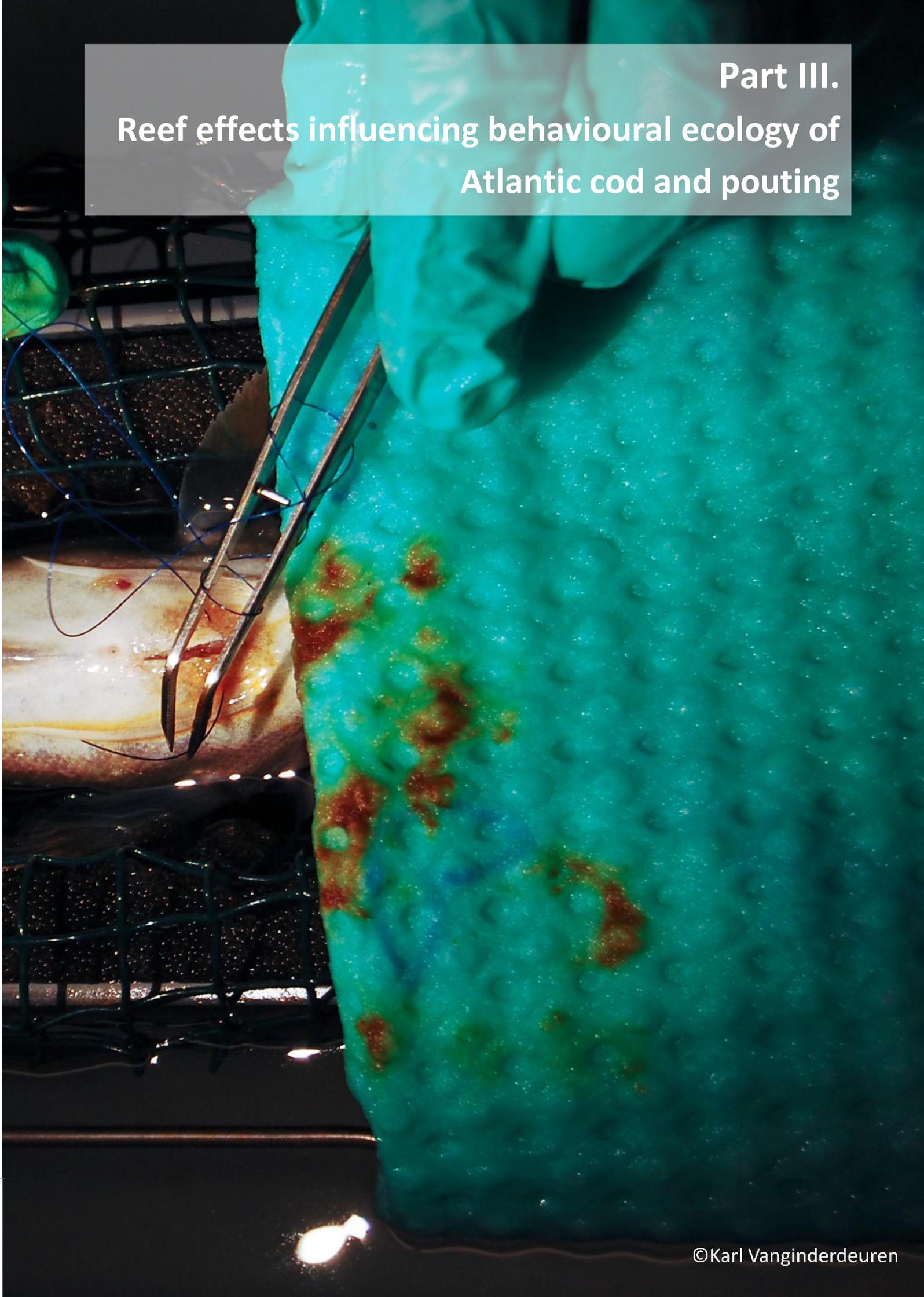
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Part III.
**Reef effects influencing behavioural ecology of
Atlantic cod and pouting**



Chapter 5

Residency, site fidelity and habitat use of Atlantic cod

Adapted from:

Reubens J, Pasotti F, Degraer S, Vincx M, In press. Residency, site fidelity and habitat use of Atlantic cod (*Gadus morhua*) at an offshore wind farm using acoustic telemetry. Marine Environmental Research.

ABSTRACT

Because offshore wind energy development is fast growing in Europe it is important to investigate the changes in the marine environment and how these may influence local biodiversity and ecosystem functioning. One of the species affected by these ecosystem changes is Atlantic cod (*Gadus morhua*), a heavily exploited, commercially important fish species. In this research we investigated the residency, site fidelity and habitat use of Atlantic cod on a temporal scale at windmill artificial reefs in the Belgian part of the North Sea. Acoustic telemetry was used and the Vemco VR2W position system was deployed to quantify the movement behaviour. In total, 22 Atlantic cod were tagged and monitored for up to one year. Many fish were present near the artificial reefs during summer and autumn, and demonstrated strong residency and high individual detection rates. When present within the study area, Atlantic cod also showed distinct habitat selectivity. We identified aggregation near the artificial hard substrates of the wind turbines. In addition, a clear seasonal pattern in presence was observed. The high number of fish present in summer and autumn alternated with a period of very low densities during the winter period.

Keywords: *Gadus morhua*, artificial hard substrates, acoustic telemetry, residency, site fidelity, habitat use, wind farms, North Sea

1. INTRODUCTION

Offshore wind energy development is the fastest growing energy technology in Europe to produce marine renewable energy (Shaw et al., 2002). In recent years offshore wind farms arose all across the North Sea (Krone, 2012; Reubens et al., 2013; van Deurs et al., 2012) and member states are planning a further monumental development in the North-East Atlantic Ocean (Wilhelmsson and Malm, 2008).

As a result thousands of wind turbines will be present in the North Sea in the near future. The foundations of these turbines form artificial hard substrates, which in time may turn into artificial reefs (so-called windmill artificial reefs, WARs). The offshore wind farms (OWFs) induce some changes in the marine environment which may influence local biodiversity and ecosystem functioning (Andersson et al., 2009). As a consequence, the OWFs have some environmental costs and benefits (Langhamer et al., 2009) including habitat alteration, changes in sediment characteristics, electromagnetic fields, underwater noise and hydrodynamics. All these ecosystem changes interact with the colonisation by epifaunal organisms; community composition of soft substrate macro- and epibenthos, demersal and benthic fish; spatio-temporal distribution and migration routes of demersal fish, seabirds and marine mammals (Degraer et al., 2012; Petersen and Malm, 2006; Reubens et al., 2013; Wilhelmsson et al., 2006). However, the ecological impacts on the marine ecosystem on the longer term are still poorly known and scientific peer-reviewed documentation is just slowly increasing (van Deurs et al., 2012).

Atlantic cod (*Gadus morhua* L., 1758) is one of the species that is affected by some of these ecosystem changes in OWFs. Reubens et al. (2013) revealed the presence of large aggregations of juvenile Atlantic cod at the foundations of wind turbines during summer and autumn. During these periods Atlantic cod exhibited crepuscular movements related to feeding activity (Reubens et al., In press-a).

Atlantic cod is a demersal fish species that occurs in the North Atlantic Ocean. It is widely distributed throughout the North Sea in a variety of habitats and is a highly valued commercial species, suffering from overexploitation (ICES, 2010). They have a flexible diel cycle in feeding activity and habitat utilization linked to spatio-temporal variations in food availability and predation risks (Clark and Green, 1990; Neat et al., 2006; Reubens et al., In press-a; Righton et al., 2001). Migratory behaviour differs between Atlantic cod groups; from sedentary cod with a very small distribution range to dispersing cod moving within large geographical areas (Robichaud and Rose, 2004). They undertake seasonal migrations between spawning, nursery and feeding grounds (Turner et al., 2002) and genetically distinct populations are present in the North Sea (Hutchinson et al., 2001). Four subgroups were found: the Bergen Bank, Moray Firth, Flamborough head and the Southern Bight. The subgroup from the Southern Bight of the North Sea is known to have winter spawning

grounds off the coasts of the United Kingdom and the Netherlands and summer feeding grounds in the southern and central North Sea (Righton et al., 2007).

As in many European countries, also Belgium invests intensively in offshore wind energy development. At present two wind farms are operational in the Belgian part of the North Sea (BPNS) and five more projects were granted a domain concession (Brabant et al., 2012). Atlantic cod is known to aggregate at these WARs (Lindeboom et al., 2011; Reubens et al., 2013) as shelter against currents or predators (Bohnsack, 1989) and increased food provisioning (Leitao et al., 2007; Reubens et al., 2011) may turn these substrates into suitable habitats for hard substrate dwelling fish. No information however is available on the possible influences of these OWFs on the temporal movement behaviour (residency, site fidelity) and habitat use of Atlantic cod.

In this research we want to:

- (1) Improve the knowledge on individual behaviour of Atlantic cod in relation to WARs. More specifically the residency and site fidelity are investigated during the summer feeding period in an OWF in the BPNS.
- (2) Investigate the small-scale habitat selectivity within an OWF. We want to distinguish whether Atlantic cod is strongly aggregated near the WARs or if they are randomly distributed on both the hard and soft substrates within a wind farm.
- (3) Investigate seasonal changes of Atlantic cod distribution near WARs in the BPNS.

2. MATERIAL & METHODS

2.1 Study site

The study was performed at the OWF of C-Power (Fig. 1). This wind farm is situated in the BPNS at the Thorntonbank, a natural sandbank 27 km offshore (coordinates WGS 84: 51°33'N – 2°56'E). The construction works started in 2008 and the wind farm should be fully operational by the end of 2013. It consists of 54 wind turbines, with two types of foundations: concrete gravity based (6 turbines) and steel jacket foundations with four legs (48 turbines). The distance between the turbines varies between 500 and 800 m. Water depth varies between 18 and 24 m and the total surface area of the wind farm is 18 km².

All Atlantic cod used in the present study were caught at two gravity based foundations (built in 2008). These foundations have a diameter of 15 metres at the seabed, at a depth of about 22.5 m at mean low water spring (MLWS). The gravity based foundations are surrounded by a scour protection layer of pebbles and rocks with a maximum radius of 18 m. The total surface area of the hard substrates (turbine foundation and scour protection together) is approximately 2043 m² (Peire et al., 2009). The surrounding soft sediment is composed of medium sand (mean median grain size 374 µm, SE 27 µm)(Reubens et al., 2009).

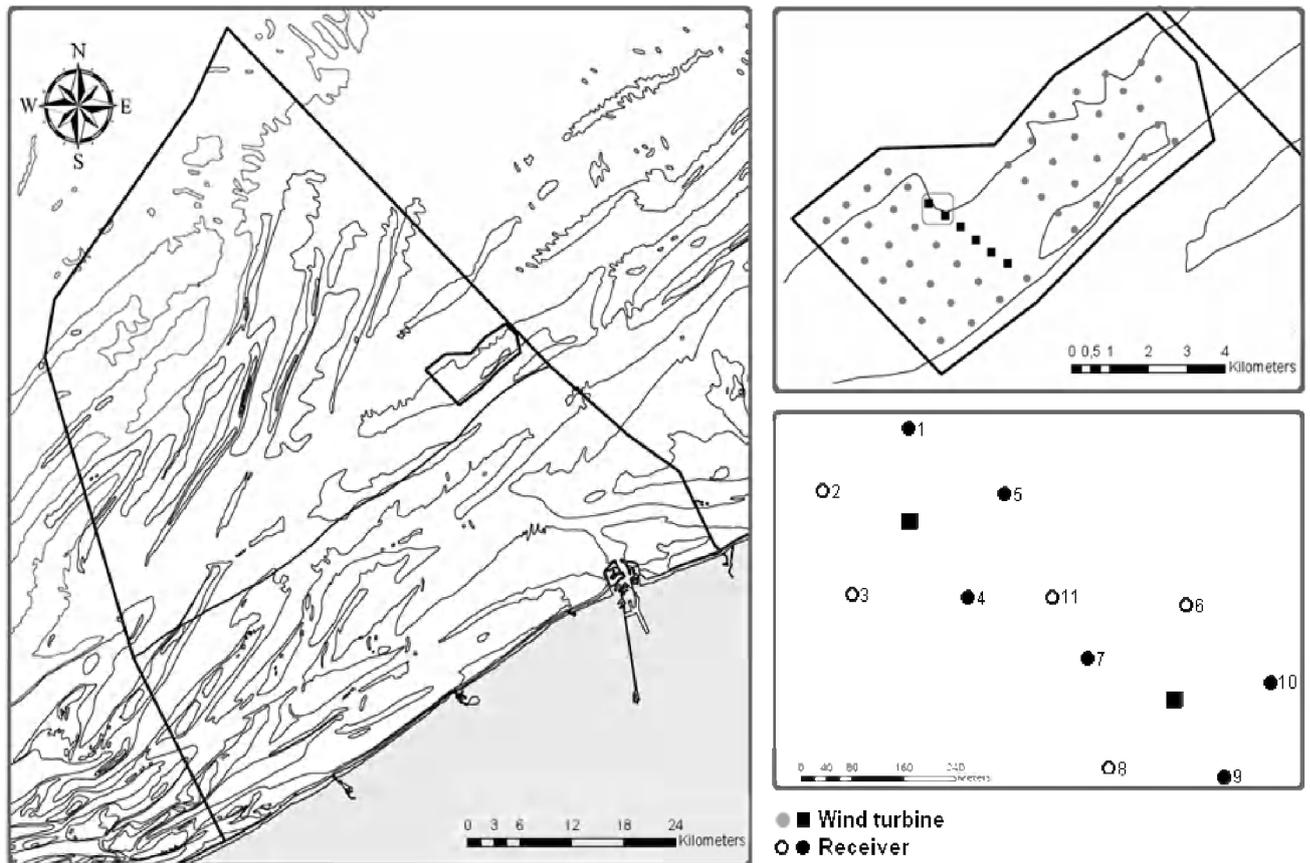


Figure 1. Overview of the Belgian part of the North Sea, with indication of the Wind farm concession area (left); wind farm layout (upper right) and receiver positions (lower right). Wind turbines are represented by grey circles (jacket foundations) and black squares (gravity based foundations). Full black circles indicate receivers that could be retrieved. All six retrieved receivers were used for the short term monitoring. For the longer term only receiver 1 & 5 were used. All fish were caught at the two wind turbines investigated.

2.2 Sampling methods

2.2.1 Residency, site fidelity and seasonality

One of the techniques used in this research is acoustic telemetry. It is an often used approach to study individual behaviour of undisturbed fish for a long period of time. In this study a design was set up to investigate residency and site fidelity and to quantify the seasonal presence of Atlantic cod at the WARs.

The Atlantic cod tracked were collected between May and July 2011 (Table 1) using hook and line gear. To minimize the probability of barotraumas, fish were hauled in slowly to allow them to release excess gas and prevent swim bladder rupture. In addition hooks without barbs were used to reduce tissue damage from hooking. After capture the individual fish were kept in an aerated water tank for two hours before surgical implantation of the acoustic transmitter (i.e. tagging). Surgical procedures were similar to those of Reubens et al. (In press-a; 2012), Arendt et al. (2001) and Jadot et al. (2006). After surgery the fish were measured and externally tagged with a T-bar anchor tag for external recognition if

recaptured. After full recovery and up to two hour observation for survival, the fish were released at their capture site. In total 22 cod specimens (age I-group) were tagged (Table 1) with Vemco coded V9-1L acoustic transmitters (Vemco Ltd., Halifax, Nova Scotia, expected lifetime of 405 days). Each transmitter has a unique ID, emitting a signal every 110 to 250 s. Fish ranged in size from 28 to 41 cm (total length). Tag weight did not exceed 2 % of the fish weight.

The Vemco VR2W acoustic monitoring system was used. Self-contained, single channel (69 kHz) submersible VR2W receivers were deployed to continuously monitor the presence of pulse-coded acoustic transmitters within their detection range. The receivers were moored on the bottom with a cast iron heating element. The receiver was attached to a polypropylene rope approximately 1 m above the seabed. The rope was connected to a subsurface buoy. When a tagged fish was detected, information on time, date and code of the specific tag were stored by the receiver. If a fish was detected, it indicates that the fish was present within the detection range of a receiver. If a fish was absent, this indicates that the fish was outside the detection range of the receivers or the signal emitted by the transmitter was blocked before it reached a receiver (e.g. by a boulder or a wind turbine foundation). In the former situation the fish had moved outside the study area (but not necessarily outside the wind farm area), in the latter the fish had moved to a position within the study area where it could not be detected.

The monitoring period was divided in two time intervals: a short term and a longer term interval. Summer-autumn residency and site fidelity were investigated during the short term, while seasonality in presence of Atlantic cod was investigated during the longer term. The receivers were placed around two WARs (Fig. 1) and recorded the presence of any acoustic transmitter within a range of 250 to 500 m. The short term monitoring period ran between May and October 2011, while the longer term monitoring period ran between May 2011 and July 2012. On October 20th 2011 four receivers (4, 7, 9 & 10) were retrieved for data analysis. The receivers 1 and 5 were retrieved on July 9th 2012. The latter were used for the longer term monitoring period, while all six receivers were used for the short term analyses. The monitoring periods are based on the dates of receiver retrieval.

Table 1. Summary of acoustic monitoring data for 22 tagged Atlantic cod. Short term monitoring ran from May until Oct 2011, while the long term monitoring ran from May 2011 until Jul 2012. Days at liberty is defined as the period between date of release and the date of last detection.

Fish no.	Length (cm)	Date released	Date first detected	Last detected (short term)	Days at liberty	Days detected	Last detected (long term)	Days at liberty	Days detected
T11	/	27/07/2011	/	/	/	/	/	/	/
T14	40	27/07/2011	01/08/2011	12/09/2011	48	43	12/09/2011	48	43
T20	37	27/07/2011	27/07/2011	20/10/2011	86	86	10/07/2012	350	348
T21	28	24/05/2011	24/05/2011	16/07/2011	54	39	16/07/2011	54	39
T22	38	07/06/2011	07/06/2011	22/08/2011	77	67	22/08/2011	77	67
T23	34	24/05/2011	24/05/2011	28/05/2011	5	5	28/05/2011	5	5
T24	36	07/06/2011	08/06/2011	20/10/2011	136	133	20/10/2011	136	133
T25	33	24/05/2011	24/05/2011	20/10/2011	150	150	10/07/2012	414	251
T26	32	24/05/2011	24/05/2011	20/10/2011	150	150	13/06/2012	387	187
T27	34	07/06/2011	11/06/2011	20/10/2011	136	132	25/12/2011	202	198
T28	34	07/06/2011	07/06/2011	20/10/2011	136	136	07/12/2011	184	164
T29	30	07/06/2011	07/06/2011	28/06/2011	22	12	28/06/2011	22	12
T30	31	07/06/2011	07/06/2011	20/10/2011	136	135	23/11/2011	170	153
T31	30	07/06/2011	07/06/2011	17/10/2011	133	115	29/10/2011	367	120
T32	37	07/06/2011	07/06/2011	07/06/2011	1	1	07/06/2011	1	1
T33	38	07/06/2011	07/06/2011	07/06/2011	1	1	07/06/2011	1	1
T34	38	07/06/2011	07/06/2011	11/06/2011	5	4	11/06/2011	5	4
T35	39	27/07/2011	27/07/2011	14/10/2011	80	44	14/10/2011	80	44
T36	41	27/07/2011	27/07/2011	20/10/2011	86	86	24/12/2011	151	151
T37	37	27/07/2011	27/07/2011	27/07/2011	1	1	27/07/2011	1	1
T38	38	27/07/2011	27/07/2011	20/10/2011	86	82	10/05/2012	289	125
T40	32	27/07/2011	27/07/2011	24/08/2011	29	29	24/08/2011	29	29

As shown in figure 1, the receiver layout in this study was not ideal. The receivers were not equally distributed around the two turbines and no perfect symmetry was obtained. At one of the turbines all three receivers were located at one side. This is the result of some logistic problems. Initially 11 receivers were deployed. Both turbines were bordered by five receivers, positioned at equal distances from each other and the turbine. An extra receiver was placed in between the two turbines. However, due to the unlikely events of storms, theft and/or damage by propellers and beam trawling only six of the receivers could be retrieved. Despite the reduced number of receivers, useful information was obtained concerning habitat use and movements of Atlantic cod near the WARs.

2.2.2 Habitat selectivity

The Vemco VR2W positioning system (VPS) was used to investigate small-scale habitat selectivity of Atlantic cod within an OWF. The study area harbours both artificial hard substrates (i.e. WARs) and soft sediments (i.e. surrounding medium sand) and to distinguish whether or not the fish were strongly aggregated towards WARs VPS data can help. VPS uses an array of VR2W receivers and synchronization transmitters to calculate the position of the transmitters. The positioning is based on the time-difference-of-arrival of an acoustic signal to at least three receivers (Espinoza et al., 2011). VPS positions are not determined in real time, but calculated using Vemco VPS software. For each calculated position the VPS provides a horizontal position error (HPE). The HPE estimates are based on the error sensitivity of the receiver layout used and calibrated for local environmental conditions (i.e. depth, salinity and water temperature) (Vemco Ltd, Nova Scotia). Based on the VPS calculated positions of the transmitters, a fish could be assigned to a specific location and thus habitat type.

2.3 Data analysis

2.3.1 Residency and site fidelity

In acoustic telemetry studies, residency and site fidelity are frequently quantified. Both terms are often used as synonyms and relate to '*presence of fish over time*'. It is the degree to which an animal returns to a specific site. However, their meaning may slightly differ depending upon the time frame investigated. In the current research residency is defined as presence over time on a daily basis, while site fidelity is defined as presence over time on an hourly basis (i.e. residency over a smaller time scale)(Schroepfer and Szedlmayer, 2006). Fish may for instance have a high residency, but low site fidelity (e.g. present every day, but only for short time during each day).

Before the acoustic data was analysed, data were filtered for spurious detections. A fish was defined as being present in the study area on a given day if it was detected at least twice on

that day. Single transmitter detections were considered false detections and removed from the analyses (Meyer et al., 2007).

For the data from the short term monitoring period a residency index was calculated, by dividing the number of days a fish was detected by the days at liberty. Days at liberty is defined as the number of days between the date of release and the date of the last detection. The residency index ranges between 0 (completely absent in the study area) and 1 (permanently present in the study area). Further, a monthly residency index was determined for each tagged fish, to investigate presence in the study area over time. The monthly residency index is defined as the number of days a fish was present during a specific month as a fraction of the total number of days in that month. The monthly residency index ranges between 0 (completely absent during a specific month) and 1 (permanently present during a specific month). Only fish observed at least once during a specific month were included in the analysis. Differences in presence were compared between periods using the non-parametric Kruskal-Wallis test.

An individual detection rate was calculated as well, to investigate site fidelity. This detection rate is defined as the number of hour bins a fish was detected within the study site as a fraction of the total time at liberty (expressed in hour bins) (Winter et al., 2010).

Analyses for residency and site fidelity were performed on data of 18 Atlantic cod as the remaining fish had insufficient detections (fish that were detected less than five days were left out). The Kruskal-Wallis tests were performed in R 2.15.1 software (www.r-project.org).

2.3.2 Habitat selectivity

To assign a fish position to a habitat type, the distance from the centre of a wind turbine foundation to the transmitter position was calculated. As the WARs extend to a distance of approximately 25 m from the centre, a fish is present at the WAR if its calculated position is less than 25 m from the centre. As such, fish positions were assigned to hard substrates, transitory or soft substrates if they were less than 25 m, 25-50 m or more than 50 m away from the centre of a wind turbine respectively.

Average relative percentages of detections were measured per distance. The relative percentage was calculated as the percentage of detections divided by the relative surface. The relative surface was calculated as a percentage of the total surface (i.e. the area covered by a distance of 150 m).

Precise position calculations are only possible if a transmitter is present within a receiver triangle. Outside the triangle there is much larger imprecision or even no position calculation possible (Vemco Ltd, Nova Scotia). As a result, only VPS estimates inside the VPS triangle (i.e. position calculations within 150 m from a turbine) were included in the analysis. Additionally only VPS estimates with an HPE value of < 25 were included in the analysis. Only fish with more than 100 calculated positions were allowed for analysis.

2.3.3 Seasonality

During the longer term interval, the seasonality in presence of Atlantic cod at the WARs was investigated. Therefore, the mean number of tagged fish present in the study area was calculated for each month. Differences in presence between periods were compared using the non-parametric Kruskal-Wallis test.

Statistical tests were performed in R 2.15.1 software (www.r-project.org). A significance level of $p < 0.05$ was used in all tests. Results are expressed as mean \pm standard deviation (SD).

Table 2. Residency and site fidelity of tagged Atlantic cod from May until October 2011. Only fish detected for more than one day are listed in the table. Site fidelity is explained by the individual detection rate. This individual detection rate is expressed as proportion of one hour time bins individual cod were detected during their time at liberty; residency is defined as the proportion of number of days a fish was detected by the days at liberty.

Fish no.	Site fidelity (%)	Residency (short term)
T14	73	0.90
T20	93	1.00
T21	37	0.72
T22	73	0.87
T23	53	1.00
T24	63	0.98
T25	85	1.00
T26	95	1.00
T27	83	0.97
T28	93	1.00
T29	13	0.55
T30	83	0.99
T31	78	0.86
T34	32	0.80
T35	46	0.55
T36	96	1.00
T38	70	0.95
T40	89	1.00

3. RESULTS

3.1 Short term monitoring period: residency and site fidelity

18 of the tagged fish were detected for 5 up to 150 days (Table 1). Most of the fish were present within the study area for an extended period of time, with many of the tagged fish still present at the end of the monitoring period (Fig. 2).

Residency was high for most fish, with 83 % having an index higher than 0.75 (Table 2). Many fish were present on a daily basis throughout almost the entire monitoring period (Fig. 2) within the study site. Further, cod showed high individual detection rates (median = 75 %). The observed proportion of hour bins that specimens were detected during their time at liberty ranged between 13 and 96 %; with half of the individuals being present more than 75 % of the time (Table 2). This indicates that many individuals showed high site fidelity.

Mean monthly residency stayed fairly constant between May and October (between 0.8 ± 0.4 and 0.9 ± 0.2). As a result, no significant differences in monthly residency could be revealed (Kruskal-Wallis, $p = 0.63$) during the short term monitoring period.

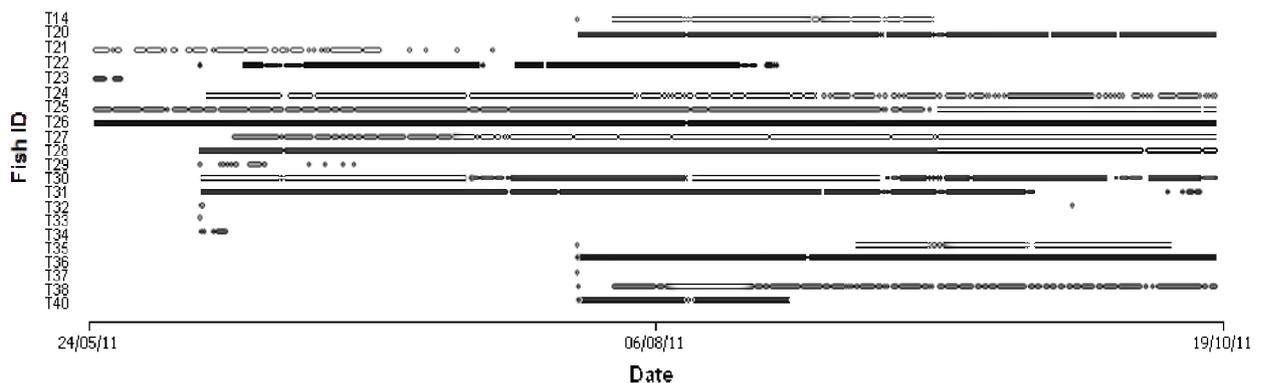


Figure 2. Overview of detections from all tagged Atlantic cod; from 24th of May until 20th of October 2011 (based on information of 6 receivers). Each line represents the detections of one fish.

3.2 Habitat selectivity

From 13 fish sufficient positions could be calculated to investigate the small scale habitat selectivity (Table 3). All fish were mainly observed between 20 and 40 m distance from a wind turbine. Almost 75 % of the relative detections were encountered on the WARs, while 97 % of the relative detections were within a 50 m range of the wind turbine. Only few detections were encountered further away (Fig. 3). This indicates that the Atlantic cod present in the study area were strongly aggregated at or close by the WARs, although the studied area was dominated by soft-bottom sediments and only small patches of hard substrates were available. Most of the detections were concentrated within this small region of hard substrates or the transitory area between hard and soft substrates.

Most of the tagged cod were observed at both wind turbines investigated. The tagged Atlantic cod were not faithful to one turbine and changed position; some movements in-between the turbines occurred.

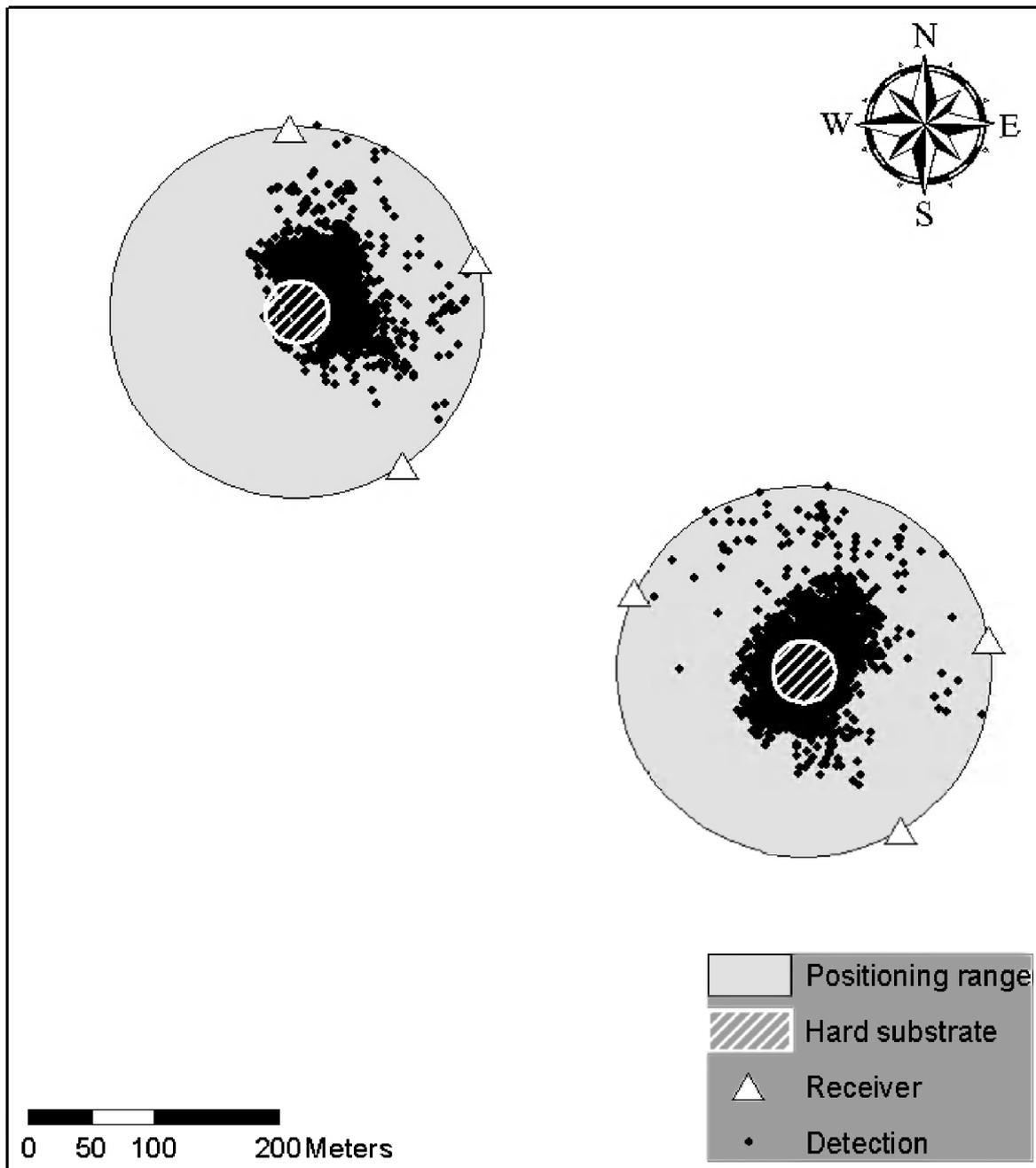


Figure 3. Overview of the calculated positions (based on 6 receivers).

Table 3. Measured distance of tagged Atlantic cod from wind turbine at detected position. Left side: number of calculated positions per distance for individual Atlantic cod; Right side: average relative percentage (\pm SE) of calculated positions per distance and cumulative percentage. Distance is calculated as distance between the calculated position and the centre of the closest wind turbine foundation. Transition between habitat types (i.e. hard, transitory and soft sediments) is indicated with a dashed line.

	T20	T22	T24	T25	T26	T27	T28	T30	T31	T35	T36	T38	T40	rel. Surface	Rel. %	SE	Cum. %
5	3	11	12	219	4	44	62	53	42	2	40	0	0	0.1	22.3	5.2	22.3
10	37	48	36	436	15	144	181	161	257	10	155	16	0	0.4	19.7	3.4	41.9
15	167	29	26	214	52	521	176	77	215	29	448	99	1	1.0	13.8	1.9	55.7
20	150	46	24	68	80	479	141	100	184	70	1750	138	0	1.8	10.5	2.4	66.2
25	136	46	29	203	97	251	193	113	165	204	1766	77	0	2.8	8.0	2.5	74.2
30	349	104	32	481	214	179	165	44	207	101	726	55	1	4.0	5.4	1.2	79.6
35	753	173	37	407	204	199	131	30	317	42	523	46	10	5.4	5.3	1.3	84.8
40	1645	264	20	93	154	133	147	10	218	14	383	116	17	7.1	5.4	2.0	90.2
45	1110	136	9	34	130	124	113	12	186	5	173	355	29	9.0	4.6	2.0	94.8
50	164	33	10	64	58	88	31	6	100	9	103	746	6	11.1	2.1	1.2	96.8
60	171	20	22	80	59	136	19	4	177	8	101	262	44	16.0	2.3	1.6	99.2
70	50	3	19	55	18	22	10	0	36	4	40	77	14	21.8	0.5	0.4	99.7
80	6	1	38	62	14	5	1	0	23	1	27	13	5	28.4	0.2	0.1	99.9
90	3	1	1	1	5	2	0	0	10	1	19	11	1	36.0	0.0	0.0	99.9
100	0	1	1	2	2	1	0	0	2	0	8	33	0	44.4	0.0	0.0	99.9
110	3	0	1	5	1	2	1	0	6	0	9	13	0	53.8	0.0	0.0	100
120	0	1	0	10	2	0	0	0	8	0	4	0	2	64.0	0.0	0.0	100
130	4	1	1	4	0	2	0	0	4	0	3	2	3	75.1	0.0	0.0	100
140	1	0	0	6	2	1	0	0	6	0	1	1	0	87.1	0.0	0.0	100
150	1	2	0	3	0	1	0	0	3	0	0	0	0	100.0	0.0	0.0	100

3.3 Longer term monitoring period: seasonal presence

During the longer term monitoring period, four (18 %) of the 22 tagged Atlantic cod were detected only the day of release. The 18 remaining fish (82 %) were detected for 5 up to 348 days (Table 1). Fish were present within the study area for an extended period of time during summer and autumn and had left the study area by the end of December or were only sporadically detected (Fig. 4). Throughout the winter months (December – March) few detections were encountered within the study area. In spring five fish returned to the WARs and three of them (Fish T25, T26 and T38) were observed for a prolonged period, although most of the fish did not return anymore after winter time.

The mean number of fish present per month was highest in July 2011 (11.2 ± 1.1) and stayed fairly constant between August and November (between 7.7 ± 2.5 and 5.6 ± 1.8). During the winter months (i.e. Dec - March) only few fish were observed (between 3.2 ± 1.3 and 1 ± 0.19). In spring, a slight increase in mean monthly numbers was noted (between 1.7 ± 0.8 and 2.2 ± 0.4). Significant differences in presence were observed between the months (Kruskal-Wallis, $p < 0.001$). Post-hoc tests revealed that mainly the summer and autumn samples (i.e. Jul – Nov) significantly differed with the winter and spring samples (i.e. Jan – Jun); confirming the seasonal trends in detection.



Figure 4. Overview of detections of all tagged Atlantic cod; from 24th of May 2011 until 9th of July 2012 (based on information of 2 receivers). Each line represents the detections of one fish.

4. DISCUSSION

Atlantic cod, as many other fish species, is liable to natural spatial and temporal patterns in movements and habitat use (Metcalf, 2006; Neat et al., 2006; Righton et al., 2007). Environmental factors play an important role in these patterns, leading to regional differences in its behaviour (Righton et al., 2001). Spatial movement differs from sedentary groups with strong site fidelity to dispersers roaming around in large geographical areas (Robichaud and Rose, 2004). Temporal movements may differ substantially between stocks and could be related to prey availability, predation pressure and abiotic factors (e.g. light

regime, prevailing currents) (Løkkeborg and Fernö, 1999; Reubens et al., In press-a; Righton et al., 2001). The present study provides important evidence concerning temporal movements and habitat use of Atlantic cod at an OWF in the BPNS.

4.1 Short term habitat use: opportunities of WARs

Reubens et al. (2013) revealed high catch rates of Atlantic cod in summer and autumn at the WARs in the BPNS. Here, residency and site fidelity were investigated in closer detail to elaborate on the behavioural ecology of Atlantic cod at this habitat during summer and autumn. Although the monitored area in this study is very limited (2 km² approximately), most of the tagged fish were present within the area for many days and showed high individual detection rates. This indicates that the tagged Atlantic cod had very restricted distribution ranges and high residency during summer and autumn. Winter et al. (2010) observed similar results in an OWF in the Netherlands; with the majority of the tagged cod exhibiting small scale movements.

Atlantic cod makes extensive migrations between feeding (i.e. in summer and autumn) and spawning grounds (i.e. in winter time) (Turner et al., 2002), but during the feeding season they may reduce their foraging movements to less than one km (Righton et al., 2001; Turner et al., 2002). The results from this study suggest that Atlantic cod uses the WARs as feeding ground. Atlantic cod is an opportunistic feeder and their diet is known to be largely determined by availability of prey (Daan, 1973). A wealth of prey species is present at the OWFs in the BPNS and the predominant prey of Atlantic cod caught near these wind turbines (Reubens et al., In press-a) are known to occur in very high densities at the WARs (Kerckhof et al., 2010a). As food is plentiful and readily available, the feeding efficiency increases near the WARs and the need for extended movements related to feeding is strongly reduced.

Other mechanisms that may stimulate site fidelity and residency near WARs are the increased protection against predators and currents (Reubens et al., In press-a; Wilhelmsson et al., 2006). At the WARs, the scour protection forms a habitat with a high complexity. The stone mattress of boulders and rocks creates an ideal shelter with many holes and crevices. In addition, there is always one side around the concrete foundations in the lee of the currents.

4.2 Habitat selectivity

The VPS study revealed that Atlantic cod are strongly attracted towards the WARs. About 97 % of the calculated positions (relative measure) were within a 50 m range from a wind turbine (note that the hard substrates extent to approximately 25 m from the wind turbine) (Table 3). Trawl data confirmed that the catch rates of Atlantic cod on soft-bottom sediments inside OWFs were very low (< 0.1 ind/km²) (Vandendriessche et al., 2012), while CPUE data from line fishing showed enhanced densities of Atlantic cod near the WAR (> 4

ind $h^{-1} fm^{-1}$ in autumn) (Reubens et al., 2013). Although no direct comparison between both fishing methods is possible, it is considered circumstantial evidence, underpinning the findings of this study.

Numerous studies have shown the potential of artificial reefs to attract and aggregate fish species (Jørgensen et al., 2002; Langhamer and Wilhelmsson, 2009; Leitao et al., 2009; Reubens et al., 2013; Reubens et al., 2011) and this aggregation effect may extend from several metres (Stanley and Wilson, 1997) to more than 100 m off an artificial reef (Soldal et al., 2002).

4.3 Longer term habitat use - Seasonal movement patterns: from feeding to spawning ground?

This study has been performed on a small spatial scale within an OWF. Movement behaviour of Atlantic cod was investigated around two WARs from a wind farm with 54 turbines. If tagged fish were no longer detected, this only signified they were not present in the study area, but could still be present inside the OWF. However, we are convinced that the two WARs investigated are representative for the entire OWF, and this for several reasons: 1) Different sampling techniques demonstrate similar results as the present study. Both line fishing and visual observations with divers revealed seasonality in catch rates at the WARs (Reubens et al., 2013 and unpublished data). During summer and autumn high catches of Atlantic cod were observed, while in winter catch rates/abundances were strongly reduced. 2) Recapture rates from an earlier tagging experiment at the WARs demonstrated that tagged Atlantic cod moved away from the WARs in winter. Recreational fishermen returned 5 of the 19 (26 %) tagged fish. Most were caught in coastal areas, indicating spatial redistribution (J. Reubens: unpublished data). 3) The majority of the wind turbines (48 out of 54) in this wind farm investigated are jacket foundations without scour protection, while the study area was at turbines with gravity based foundations. Preliminary results indicate that the jacket foundations are less attractive to Atlantic cod compared to the gravity based foundations (J. Reubens: unpublished data).

Atlantic cod exhibited a clear seasonal pattern in presence. Fish were present at the WARs for an extended period of time during the summer. In autumn the numbers decreased and in winter time almost all fish had left the study area. Although some fish returned to the WARs, most were no longer encountered. Comparable results were found in an OWF in the Netherlands (Winter et al., 2010). Many of the tagged cod had left the OWF by winter, although some stayed throughout the winter season.

As mentioned before, Atlantic cod makes extensive migrations from feeding (i.e. in summer and autumn) to spawning grounds (i.e. in wintertime) (Turner et al., 2002). To our knowledge however, there are no known spawning locations in the Belgian part of the North Sea and

Righton et al. (2007) showed that Atlantic cod from the Southern Bight of the North Sea has some spawning areas along the coasts of the United Kingdom and the Netherlands. Thus, the seasonal pattern in presence at the WARs might be related to spawning migrations.

Only few of the tagged cod returned to the WARs in spring 2012. Fish may no longer be interested any more to this type of substrate due to changes in their life history behaviour. Predator-prey relationships alter with age, related to prey size preferences (Daan, 1973). Younger Atlantic cod mainly forage on smaller crustaceans (e.g. amphipods, small crabs) which are readily available at the WARs. Older individuals change to a fish dominated diet (Daan, 1973). In addition, older fish are less vulnerable to predation themselves as cannibalism and predation by other fish species does not longer occur. As a result, older Atlantic cod are less dependent of protective habitat.

For younger ages, predation dominates Atlantic cod mortality, while fishery takes over at older ages (Link et al., 2009). This might be the second reason for the low return rate after winter time. Inside the Belgian offshore wind farms no fishery activities are allowed, enhancing the survival rate of cod present in these areas. Once they left the areas, they are more vulnerable to fisheries (both commercial and recreational). Julliard et al. (2001) revealed that fisheries mortality of the 0-group Atlantic cod is negligible, but that it is high for older fish. More than 60 % of the 2 to 4- year-old Atlantic cod in the North Sea are caught annually by fisheries (ICES, 2013). This indicates that fisheries mortality may influence fish survival considerably and may hence have artificially reduced the probability of fish to return to the wind farm after winter migration.

It can be concluded that Atlantic cod demonstrates strong residency and high individual detection rates during summer and autumn at the WARs investigated, which is probably related to the use of this habitat as feeding ground. Within the OWF, Atlantic cod shows distinct habitat selectivity behaviour and is strongly attracted towards the artificial hard substrates. In addition a seasonal pattern in presence at the WARs is observed. The high residency during summer and autumn alternates with a period of very low presence during winter time.

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Chapter 6

Diurnal activity and movement patterns of Atlantic cod

Adapted from:

Reubens J, De Rijcke M, Degraer S, Vincx M, In press. Diel variation in feeding and movement patterns of juvenile Atlantic cod at offshore wind farms. *Journal of Sea Research*.

ABSTRACT

Atlantic cod (*Gadus morhua*) is a commercially important fish species suffering from overexploitation in the North-East Atlantic. In recent years, their natural environment is being intensively altered by the construction of offshore wind farms in many coastal areas. These constructions form artificial reefs influencing local biodiversity and ecosystem functioning. It has been demonstrated that Atlantic cod is present in the vicinity of these constructions. However, empirical data concerning the diel activity and feeding behaviour of Atlantic cod in the vicinity of these artificial reefs is lacking. Atlantic cod has a flexible diel activity cycle linked to spatio-temporal variations in food availability and predation risk. In this study we integrated acoustic telemetry with stomach content analysis to quantify diel activity and evaluate diel feeding patterns at a windmill artificial reef (WAR) in the Belgian part of the North Sea. Atlantic cod exhibited crepuscular movements related to feeding activity; a 12 h cycle was found and the highest catch rates and stomach fullness were recorded close to sunset and sunrise. It is suggested that the observed diel movement pattern is related to the prey species community and to predation pressure. Foraging at low ambient light levels (i.e. at dusk and dawn) probably causes a trade-off between foraging success and reducing predation pressure. Fish did not leave the area in-between feeding periods. Hence other benefits (i.e. shelter against currents and predators) besides food availability stimulate the aggregation behaviour at the WARs.

Keywords: diel activity, *Gadus morhua*, feeding, artificial hard substrates, offshore wind farms

1. INTRODUCTION

Atlantic cod (*Gadus morhua* Linnaeus, 1758) is a demersal fish species occurring throughout the North Atlantic Ocean (Froese and Pauly, 2012). It has a considerable commercial value and many populations have been heavily exploited for several centuries (Serchuk and Wigley, 1992). This resulted in critically low population levels for many stocks in recent years (ICES, 2010; Svedäng and Bardon, 2003). Due to its commercial importance and its dwindling stocks, the life history traits (Lund et al., 2011; Olsen et al., 2004), abundances (Rose and Kulka, 1999; Svedäng and Bardon, 2003), movements (Lindholm et al., 2007; Metcalfe, 2006; Svedäng et al., 2007) and feeding behaviour (Adlerstein and Welleman, 2000) of Atlantic cod have been documented in many studies over a wide range of spatial and temporal scales using a variety of techniques and approaches.

However, natural behaviour, abundances and movements of Atlantic cod may be influenced by offshore human activities. Solid structures (e.g. gas platforms (Lowe et al., 2009), wind turbines (Reubens et al., 2011) and wave power foundations (Langhamer et al., 2009)) have been placed on the seabed all around the world and can be classified as artificial reefs. These artificial reefs have some environmental costs and benefits (Langhamer et al., 2009) which may influence local biodiversity and ecosystem functioning (Andersson et al., 2009). Numerous offshore wind farms are currently being constructed in the North Sea and research on the effects of these Windmill Artificial Reefs (further referred to as WARs) on the surrounding marine environment is required. Some demersal fish species for instance, are likely to be attracted to the WARs as shelter against currents or predators (Bohnsack, 1989) and increased food provisioning (Leitao et al., 2007; Reubens et al., 2011) may turn these substrates into suitable habitats for hard substrate dwelling fish.

Reubens et al. (2013) revealed the presence of large aggregations of juvenile Atlantic cod at WARs in the Belgian part of the North Sea (BPNS) during summer and autumn. However, empirical data concerning the reason why this species seems to be attracted by the reefs is unclear. Information on the diel movements and feeding behaviour of Atlantic cod in the vicinity of WARs is still lacking. The diel variation needs to be taken into account as this might shed light on the true added value of WARs. Next, Atlantic cod are also known to have a flexible diel cycle in feeding activity and habitat utilization which may differ between life stages, season and habitat (Clark and Green, 1990; Keats and Steele, 1992; Neat et al., 2006). It is often assumed that these differences in diel activity patterns are linked to spatio-temporal variations in predation pressure and food availability (Løkkeborg and Fernö, 1999; Righton et al., 2001).

The wind farm under consideration harbours a diverse epifaunal community with high species abundances (Kerckhof et al., 2010b). Many of these epifaunal species are potential prey for juvenile Atlantic cod (Froese and Pauly, 2012). Several natural predators of Atlantic cod are also present in the area. The harbour porpoise (*Phocoena phocoena* Linnaeus, 1758)

is present year round and may reach high abundance during late winter, early spring. The harbour seal (*Phoca vitulina* Linnaeus, 1758), grey seal (*Halichoerus grypus* Fabricius, 1791) and the white-beaked dolphin (*Lagenorhynchus albirostris* Gray, 1846) are also observed in Belgian waters, be it in much lower numbers compared to harbour porpoises (Haelters et al., 2011). All types of fisheries are excluded in the wind farm, leading to less human disturbance of the associated fish aggregations. Therefore, this wind farm provides an ideal opportunity to investigate the diel behaviour of an Atlantic cod aggregation in relation to food availability and predator pressure. However, directly observing the behaviour of marine fish in the wild is logistically very difficult. As a result, other methods are essential to infer fish behaviour (Hall et al., 1995). In this study we integrated acoustic telemetry with stomach content analysis. The former method was used to empirically quantify diel movement behaviour, while the latter is used to evaluate diel feeding patterns. Several questions were addressed: (1) do Atlantic cod at WARs exhibit predictable diel activity and movement patterns? (2) is there a diel pattern in feeding rates and prey composition?

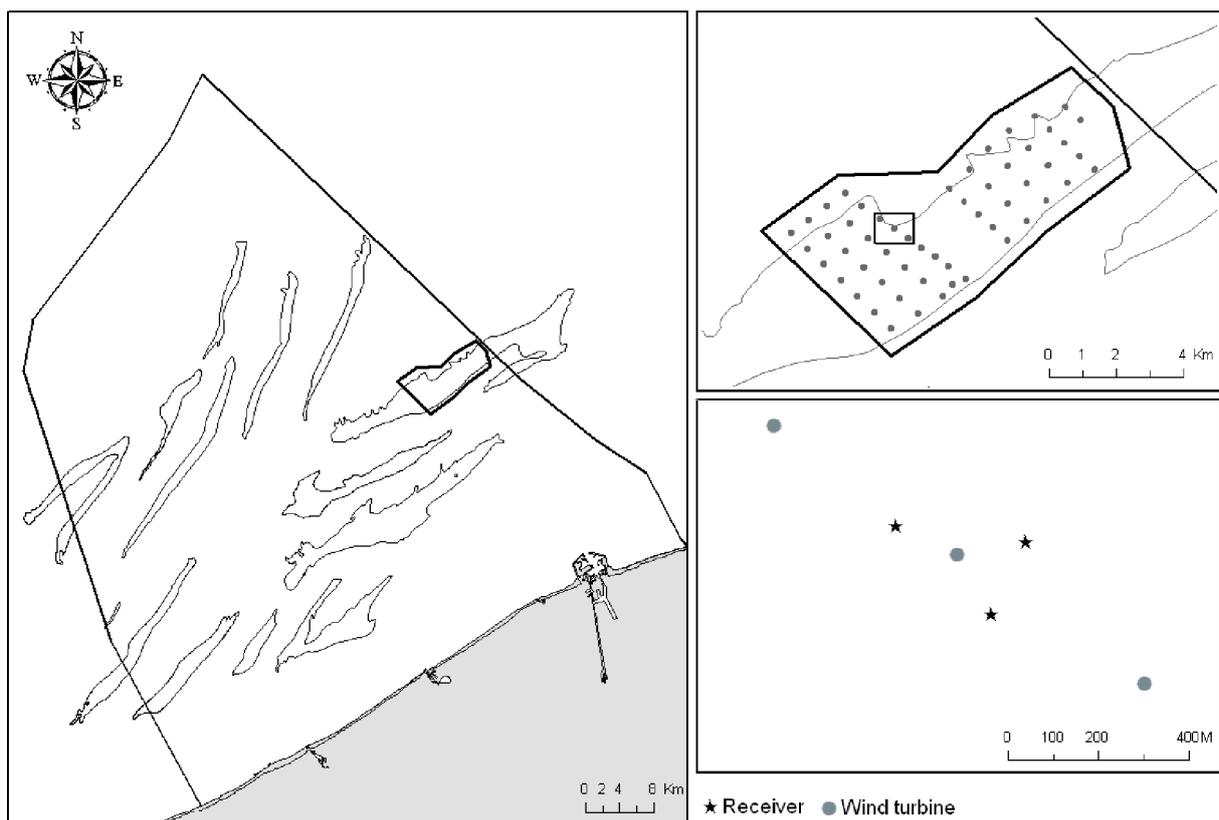


Figure 1. Overview of the Belgian part of the North Sea, with indication of the Wind farm concession area (left); wind farm layout (upper right) and receiver positions (lower right).

2. MATERIAL & METHODS

2.1 Study site

The wind farm under consideration is situated in the BPNS at the Thorntonbank (Fig. 1), a natural sandbank 27 km offshore (coordinates WGS 84: 51°33'N – 2°56'E). Two types of foundations are present in this farm: concrete gravity based and steel jacket foundations. Both function as WARs. All Atlantic cod used in the present study were caught at gravity based foundations. These foundations have a width of 15 metres at the seabed, at a depth of about 22.5 m at mean low water spring (MLWS). The gravity based foundations are surrounded by a scour protection layer of pebbles and rocks with a total diameter of 51 m (2043 m²) (Peire et al., 2009). The surrounding soft sediment is composed of medium sand (mean median grain size 374 µm, SE 27 µm)(Reubens et al., 2009).

2.2 Sampling methods

2.2.1 Acoustic telemetry

To quantify the diel movement pattern of Atlantic cod at the WARs, the Vemco VR2W acoustic monitoring system was used. In this system self-contained, single channel (69 kHz) submersible VR2W receivers were used to detect the signals of pulse-coded acoustic transmitters (Vemco V9-1L). Each transmitter has a unique ID, emitting a signal every 110 to 250 s.

The Atlantic cod tracked at the WARs, were collected between May and July 2011 (Table 1) in the study area using hook and line gear. After capture the individual fishes were kept in an aerated water tank for 2 hours before surgical implantation of the transmitter (i.e. tagging). Surgical procedures were similar to those of Reubens et al. (2012), Arendt et al. (2001) and Jadot et al. (2006). Prior to tagging, the fish were anaesthetized in a 0.3 ml l⁻¹ 2-phenoxyethanol solution. Following anaesthesia, (i.e. fish showing no reaction to external stimuli, slow opercular rate and loss of equilibrium (McFarland and Klontz, 1969)), the fish were placed, ventral side up, in a V-shaped support. Most of the body, except the ventral side, stayed in the water and a continuous flow of aerated water was pumped over the gills to avoid dehydration and provide continuous oxygenation. A small incision (15-22 mm) was made on the mid-ventral line and an acoustic transmitter was inserted in the visceral cavity. The incision was closed with two sutures (polyamide monofilament, DS19 3/0). In total, 22 cod specimens were tagged. The fish were further externally tagged with a T-bar anchor tag. After full recovery and up to two hour observation for survival, the fish were released at their capture site. Data were used for analysis from one day post-release as fish might not exhibit normal behaviour the first hours after release (Bridger and Booth, 2003). The acoustically tagged Atlantic cod specimens were tracked with three automated acoustic receivers. The receivers were placed around one WAR (Fig. 1) and recorded the presence of

any acoustic transmitter within a range of 250 to 500 m. On the 20th of October 2011 the receivers were retrieved for data analysis.

Table 1. General information of the acoustic monitoring (May-June 2011) at a windmill artificial reef in the Belgian part of the North Sea of 22 tagged Atlantic cod. Time at liberty indicates the number of days between the first and the last detection. ^{NA} Not applicable.

Fish ID	Length (cm)	Release date	Date first detected	Date last detected	Time at liberty	days detected
T21	28	24/05/2011	24/05/2011	16/07/2011	54	43
T23	34	24/05/2011	24/05/2011	28/05/2011	5	5
T25	33	24/05/2011	24/05/2011	20/10/2011	150	150
T26	32	24/05/2011	24/05/2011	20/10/2011	150	150
T22	38	07/06/2011	07/06/2011	02/08/2011	77	64
T24	36	07/06/2011	08/06/2011	20/10/2011	136	135
T27	34	07/06/2011	11/06/2011	02/10/2011	118	103
T28	34	07/06/2011	07/06/2011	20/10/2011	136	136
T29	30	07/06/2011	07/06/2011	28/06/2011	22	13
T30	31	07/06/2011	07/06/2011	20/10/2011	136	136
T31	30	07/06/2011	07/06/2011	17/10/2011	133	110
T32	37	07/06/2011	07/06/2011	01/10/2011	117	2
T33	38	07/06/2011	07/06/2011	07/06/2011	1	1
T34	38	07/06/2011	07/06/2011	11/06/2011	5	5
T11	/	27/07/2011	/	/	NA	0
T14	40	27/07/2011	01/08/2011	12/09/2011	48	43
T20	37	27/07/2011	27/07/2011	02/10/2011	68	67
T35	39	27/07/2011	27/07/2011	14/10/2011	80	28
T36	41	27/07/2011	27/07/2011	19/10/2011	85	74
T37	37	27/07/2011	/	/	NA	0
T38	38	27/07/2011	27/07/2011	18/10/2011	84	71
T40	32	27/07/2011	27/07/2011	24/08/2011	29	29

2.2.2 Stomach content analysis

To quantify the feeding rate of Atlantic cod and prey composition in their diet on a diel base, line fishing was conducted. A 24 h sampling campaign was performed on the 29th and 30th of July 2010 at a WAR. Sampling was performed for 30 min at 3 h time intervals. Times of sunrise and sunset were recorded at 04h06 and 19h33 (Coordinated universal Time) respectively. Angling (hooks: Arca, size 4; bait: *Arenicola marina*) was performed 1 to 10 metres away from a turbine (i.e. within the erosion protection layer radius) just above the bottom of the seabed, assuring catching individuals hovering at the WAR. The fish were

measured (total length), weighed (wet weight) and stomachs were removed and preserved in an 8% formaldehyde-seawater solution. All food components in the stomachs were identified to the lowest possible taxonomic level. Crabs that could not be identified to the genus level were named as 'Brachyura sp.'. Wet weight, dry weight (60 °C for 48 h) and ash free dry weight (500 °C for 2 h) were measured for all separate food contents in each stomach. No analysis was performed if stomachs were not preserved appropriately. In total 305 stomachs were used for analysis. Atlantic cod length varied between 25.2 and 53.7 cm (mean \pm SD was 35.7 ± 4.2 cm).

The setup for acoustic telemetry and line fishing were not organised at the same turbine. Telemetry was performed at wind turbine D5, while fishing was organised at D1. In 2010 a test study was running at D5 to analyse the possibility to perform acoustic telemetry. It was decided not to perform the 24h fishing sampling campaign at the same wind turbine, to minimize the chance of catching tagged fish. However, D1 is in close proximity to D5 (approximately 2 km), it is also a gravity based foundation and environmental parameters are very much comparable.

2.3 Data analysis

2.3.1 Acoustic telemetry

First, the data were filtered for spurious detections. A fish was defined as being present in the study area on a given day if it was detected at least two times on that day. Single transmitter detections were defined as false detections and removed from the analyses (Meyer et al., 2007).

The Fast Fourier Transformation (FFT) time series analysis was used to investigate periodicity in the behaviour. FFT breaks down a time series into the sum of its sinusoidal components. It describes fluctuations in a time series by comparing them to sinusoids. Frequencies of dominant patterns are identifiable as peaks (Bloomfield, 2004; Meyer et al., 2007). In FFT algorithms, the number of cases must be equal to a power of 2. If this is not the case, additional computations have to be performed (Statistica, Statsoft). The input dataset was not padded, exact length was used. Prior to FFT the detections of each fish were pooled into hourly bins. FFT was applied on data of 18 Atlantic cod as the remaining fish had insufficient detections for analysis (fish that were detected less than five days were left out). We further investigated possible diel patterns in detections linked to the photoperiod. Therefore, detections of each specimen were binned by period (i.e. sunrise, day, sunset and night) and daily average detections (weighted for the length of each period throughout the study) were compared between periods using the non-parametric Kruskal-Wallis test (data did not allow using parametric tests).

Sunrise and sunset information was obtained by the Royal Observatory of Belgium. The FFT was performed in Statistica (version 7.0, Statsoft, Tulsa, Oklahoma), while Kruskal-Wallis tests were performed in R 2.15.1 software (www.r-project.org).

2.3.2 Stomach content analysis

To investigate the diel pattern in feeding rates a stomach content fullness index (I_F) was calculated for each stomach of Atlantic cod. $I_F = 10\,000cL^{-3}$, where c is the stomach content mass (wet weight, g) and L is the fish length (total length, cm). The I_F adjusts for variation in fish size (Darbyson et al., 2003). Only non-empty stomachs were used in the analysis as regurgitation could have occurred while fish were being hauled. The non-parametric Kruskal-Wallis test was used to compare stomach content fullness between the 3 h sampling intervals (i.e. fishing batches). Statistical analysis was performed in R 2.15.1 software (www.r-project.org). Data did not allow using parametric tests.

Further, prey composition of the diet was also examined by time of the day. Dietary composition was assessed by gravimetric abundance (ash-free dry weight) of each prey species for each batch. Prey composition was compared within and between time frames using non-metric multi-dimensional scaling and was statistically tested using a randomization test (analysis of similarity) based on permutations of the similarity matrix (Clarke and Gorley, 2006). Prior to analysis the community abundance data were standardized (De Crespín de Billy et al., 2000) and a similarity matrix was constructed using the Bray-Curtis index of similarity. Statistical analyses were performed using the Plymouth Routines in Multivariate Ecological Research (PRIMER) package, version 6.1.6 (Clarke and Gorley, 2006). A significance level of $p < 0.05$ was used in all tests. Results are expressed as mean \pm standard error (SE).

3. RESULTS

3.1 Diel movement patterns

The acoustic monitoring ran between May and October 2011. The receivers were recovered on 20/10/2011. The receivers detected 20 of the 22 acoustically tagged Atlantic cod. The total number of days on which a fish was detected ranged between 1 and 150 days (median 53.5 days) (Table 1).

The FFT analysis, done on 2048 h of continuous observations, revealed a clear diel cycle in movements of Atlantic cod (Fig. 2). The dominant peak in detections disclosed a 12 h periodicity. A secondary 6 h peak was observed as well.

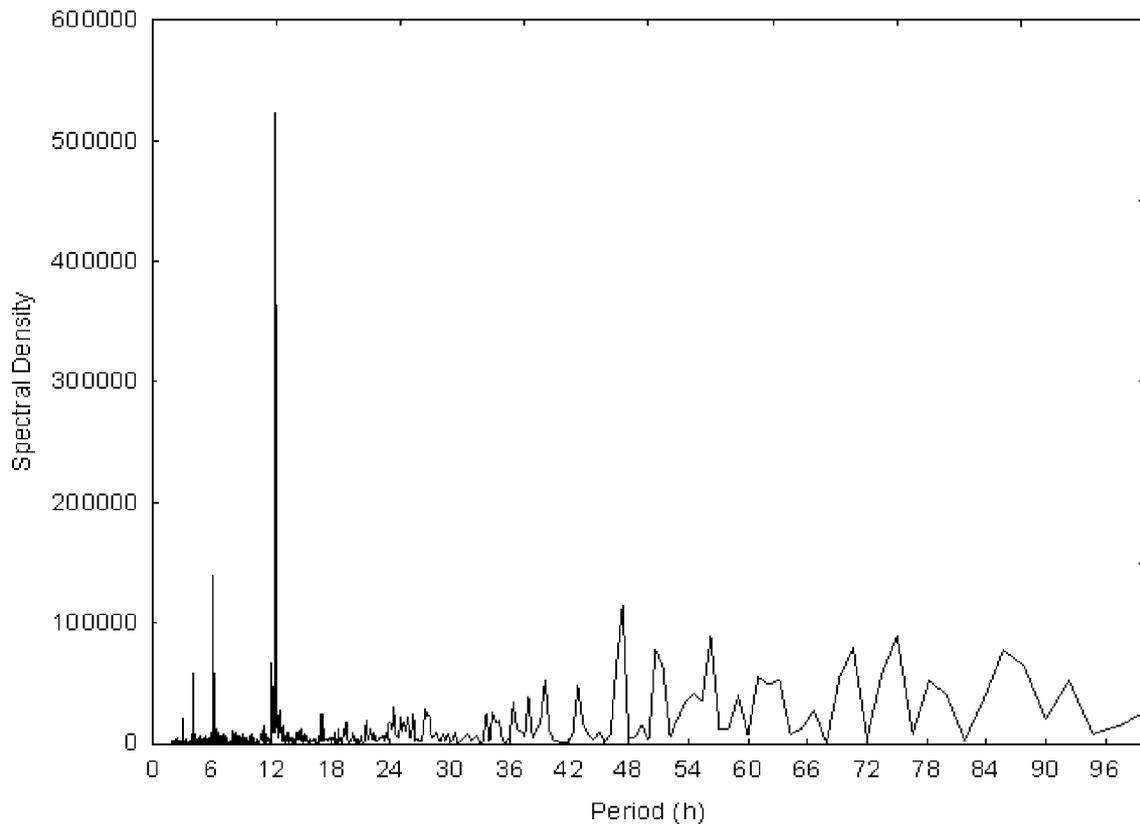


Figure 2. Fast Fourier Transformation from 2048 continuous hours of detections of 18 Atlantic cod. Peaks indicate the periodicity of dominant cycles.

We found evidence that Atlantic cod occurs in the vicinity of the WAR during the whole diurnal cycle. Each acoustically tagged fish had comparable average daily detections per time bin (Table 2). The fish were detected both day and night, indicating that they do not leave the WAR area (on a daily basis), even not in periods of lower detection rates. Pooling all fish, no significant difference in detections was present between the time bins (Kruskal-Wallis, $p = 0.23$). However, some individual variability in temporal patterns was observed. For many fish, the average number of detections did not vary significantly between periods; while for other individuals significant differences were present, mainly between day/night and dusk/dawn (Kruskal-Wallis post-hoc multiple comparison tests)(Table 2). This is in line with the results from the FFT analysis.

Further, daily detection rates vary highly between individual specimens.

Table 2. Average daily detections per time bin (weighted for the length of each period) from 18 acoustically tagged Atlantic cod. Differences in detections between groups were compared using the non-parametric Kruskal-Wallis test.

	χ^2	df	p-value	Average \pm SE			
				dawn	day	dusk	night
All	4.3125	3	0.23	90.9 \pm 5.3	84.8 \pm 4.2	94.8 \pm 5.3	73.6 \pm 3.4
T14	2.0344	3	0.57	5.0 \pm 0.7	5.1 \pm 0.4	6.3 \pm 0.9	4.9 \pm 0.4
T20	0.8083	3	0.85	11.3 \pm 1.1	9.9 \pm 0.9	12.5 \pm 1.3	10.0 \pm 0.9
T21	8.0197	3	0.05*	3.9 \pm 0.8	3.3 \pm 0.6	6.5 \pm 1.7	5.6 \pm 0.9
T22	9.3109	3	0.03*	7.1 \pm 1.4	6.7 \pm 1.0	6.9 \pm 1.4	4.0 \pm 0.7
T23	1.5737	3	0.67	4.2 \pm 3.5	5.7 \pm 3.3	3.1 \pm 2.0	5.1 v 2.3
T24	10.0138	3	0.02*	6.3 \pm 0.7	6.0 \pm 0.5	5.9 \pm 0.7	5.2 \pm 0.5
T25	17.2766	3	<0.01*	11.5 \pm 0.9	14.2 \pm 0.7	13.7 \pm 1.0	10.3 \pm 0.6
T26	5.3184	3	0.15	17.3 \pm 0.9	14.9 \pm 0.6	16.4 \pm 0.9	13.9 \pm 0.5
T27	31.1179	3	<0.01*	3.5 \pm 0.6	4.8 \pm 0.6	4.7 \pm 0.7	3.0 \pm 0.4
T28	1.7774	3	0.62	15.1 \pm 0.9	13.3 \pm 0.6	15.6 \pm 1.0	13.7 \pm 0.6
T29	9.9463	3	0.02*	3.0 \pm 2.4	1.3 \pm 0.5	0.3 \pm 0.3	1.9 \pm 1.2
T30	1.8289	3	0.61	9.7 \pm 0.8	8.9 \pm 0.5	8.9 \pm 0.7	7.8 \pm 0.5
T31	5.9133	3	0.12	15.0 \pm 1.2	14.4 \pm 0.9	13.8 \pm 1.1	11.1 \pm 0.7
T34	1.9408	3	0.58	4.7 \pm 4.1	3.0 \pm 1.5	6.1 \pm 6.1	1.6 \pm 0.7
T35	8.6481	3	0.03*	1.9 \pm 0.6	1.4 \pm 0.5	3.3 \pm 1.3	1.4 \pm 0.3
T36	12.5062	3	0.01*	3.2 \pm 0.6	2.0 \pm 0.3	4.2 \pm 0.7	2.5 \pm 0.3
T38	8.7376	3	0.03*	7.7 \pm 1.3	6.8 \pm 0.9	7.4 \pm 1.2	4.5 \pm 0.8
T40	0.1952	3	0.98	10.2 \pm 1.5	9.3 \pm 1.0	10.4 \pm 1.6	9.9 \pm 0.8

3.2 Diel pattern in feeding rates and prey composition

In total 308 Atlantic cod were caught over the eight fishing batches. Average length was 35.7 \pm 4.2 cm, indicating they belong to age group I and II (ICES Fishmap, <http://www.ices.dk/marineworld/fishmap/ices/>). Catch per unit effort (CPUE) was highest immediately before sunset and after sunrise (Table 3). CPUE decreased significantly during night time (mean = 11 \pm 3) compared to day time (mean = 26 \pm 2) (Mann-Whitney U-test, p = 0.04). 40 of the 305 stomachs analysed were empty (13.1 %).

Table 3. Catch per unit effort per fishing batch, sunset and sunrise were recorded at 19h33 and 04h06 (UTC) respectively

Batch	Sampling time (UTC)	CPUE (ind h ⁻¹ fm ⁻¹)	Grouping
B1	09:10 - 09:40	21.5	day
B2	12:00 - 12:25	27	day
B3	14:55 - 15:30	21	day
B4	17:55 - 18:20	29.4	day
B5	21:05 - 21:40	9.89	night
B6	00:25 - 00:55	17.5	night
B7	03:05 - 03:40	5.71	night
B8	06:00 - 06:25	31.2	day

The mean fullness index was highest immediately after sunset (batch 5: 0.94 ± 0.33) and sunrise (batch 8 and 1: 0.86 ± 0.11 and 0.90 ± 0.13 respectively). Figure 3 reveals that stomach fullness followed a clear trend. Mean stomach fullness peaked immediately after sunset and sunrise, followed by a gradual decrease. After reaching a minimum during midday/midnight stomach fullness gradually increased towards twilight periods. A significant difference in fullness index was present between the different batches (Kruskal-Wallis, $p = 0.007$). Post-hoc analysis revealed that B3 and B8 differed strongest in stomach fullness.

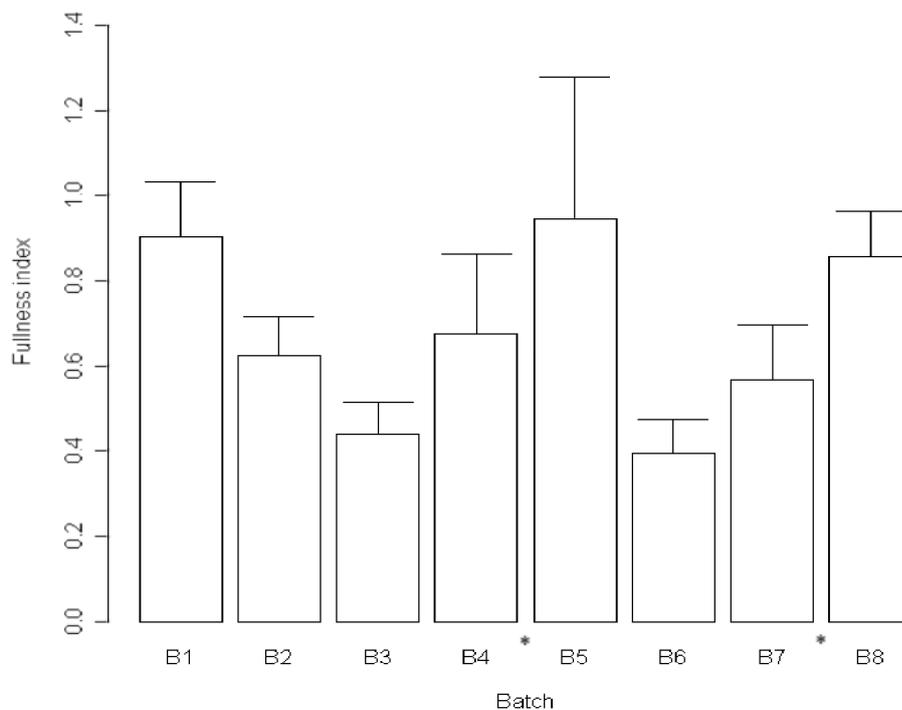


Figure 3. Fullness index (mean + SE) during each fishing batch. * indicates the moment of sunset and sunrise respectively.

The diet of Atlantic cod showed a wide variety in prey species, many of which are epifaunal species or associated with hard substrates. The predominant prey species in the diet were *Pisidia longicornis*, *Brachyura* sp., *Liocarcinus* spp. and *Actiniaria* sp. Some amphipod species (i.e. *Jassa herdmani*, *Phtisica marina* and *Monocorophium acherusicum*) had a high frequency of occurrence as well and reached high abundances, but contributed less to the total prey biomass. Within each batch, a broad diversity of prey species was present. Stomach content did not differ significantly between batches (Anosim, $p = 0.27$, $R = 0.007$).

P. longicornis had a high frequency of occurrence in all batches. *Liocarcinus* spp. and *Brachyura* sp. occurred more in stomachs of Atlantic cod caught during the night, while *J. herdmani* was more often present in day time samples. A comparable trend was present in the relative contribution of the predominant preys to the stomach content weight in each batch (Fig. 4).

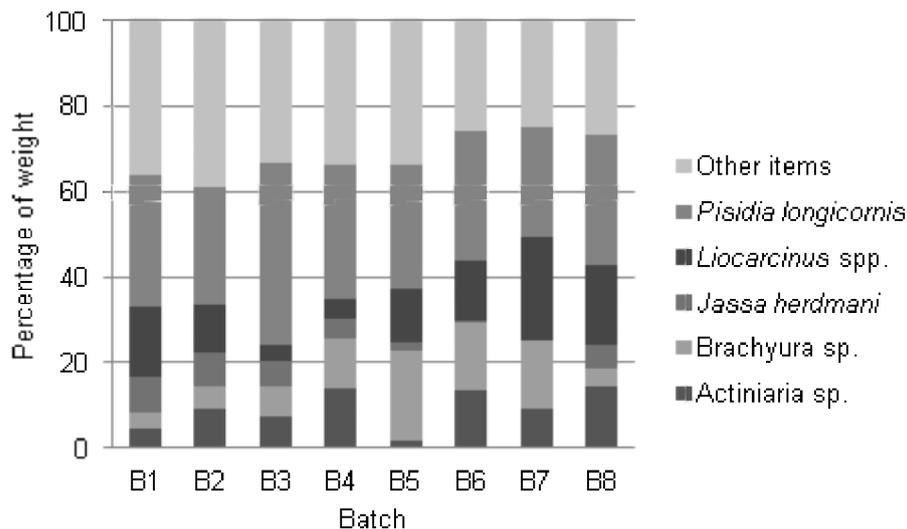


Figure 4. Average relative weight of dominant prey species in the stomachs of Atlantic cod during each batch.

4. DISCUSSION

Diurnal movement patterns

The Vemco VR2W acoustic monitoring system was used to record the presence of fish equipped with an acoustic transmitter within a certain distance of a receiver. If a tagged fish was detected, this indicates that the fish was present within the detection range of that specific receiver. If a fish was absent, this indicates that the fish was outside the detection range of the receiver or the signal emitted by the transmitter was blocked before it reached the receiver (e.g. by a boulder or a wind turbine foundation). In the former situation the fish had moved outside the study area, in the latter the fish had moved to a position within the study area where it could not be detected. In this way, the presence/absence data obtained by the system can be used to measure fish movements.

FFT analysis of the data revealed a dominant 12 hour peak in detections at the WAR, indicating crepuscular movements. If this 12 hour periodicity is linked to the fullness index, it clearly shows that Atlantic cod is most active at the WARs during twilight periods (Fig. 5). During these periods they actively forage, resulting in enhanced food intake and stomach fullness. As a consequence, they have the highest chance to be caught by line fishing during twilight, which is consistent with the CPUE data (Table 3).

Our results are in agreement with the results from similar studies on North Sea cod in which also morning and evening peaks in stomach fullness were found, reflecting intensive feeding at sunrise and sunset (Adlerstein and Welleman, 2000; Rae, 1967). However, this is not always the case. Atlantic cod is known to have a flexible diel feeding activity cycle (Helfman, 1993) that may differ between regions. Clark and Green (1990) found that 3-year-old Atlantic cod in Newfoundland were nocturnally active during summer, switching to daytime activity in autumn. Løkkeborg (1989) found that Atlantic cod exhibited a morning and afternoon peak in activity. Rae (1967) concluded that Atlantic cod in the North Sea displays crepuscular feeding patterns, while Daan (1973) found no consistent pattern.

Prey availability and feeding behaviour

Patterns in diel feeding activity have been demonstrated to be influenced by the activity pattern of prey species and the predation pressure (Clark and Green, 1990; Løkkeborg and Fernö, 1999; Neat et al., 2006). If important prey species have a specific diel activity cycle, Atlantic cod is expected to follow this cycle. Meanwhile, Atlantic cod need to minimize the predation risk themselves. Therefore active foraging will coincide with light conditions that maximize the feeding success in relation to predation pressure (Helfman, 1993). Adlerstein & Welleman (2000) for instance, found that the most intensive feeding period of Atlantic cod coincided with the diel migration of sandeels, a dominant prey in their diet.

Atlantic cod is an opportunistic feeder and their diet is known to be largely determined by availability (Daan, 1973), which is in agreement with the present findings. The predominant preys in this study (i.e. *Pisidia longicornis*, *Liocarcinus* spp., *Actinaria* sp. and *Jassa herdmani*) are known to occur in high densities (up to 13,000 and 4,000 ind/m² for *J. herdmani* and *P. longicornis* respectively) at the WAR studied (Kerckhof et al., 2010a).

The observed diel feeding activity pattern of Atlantic cod in the present study is supposed not to be linked to the activity pattern of one or several dominant prey species. Although some trends are present in relative contribution of dominant prey species in the diet among batches (Fig. 4) none of these can be clearly linked to the activity cycle of Atlantic cod. There were no significant differences in stomach contents between the batches and a large variety of potential prey species were present in high densities, both day and night (Kerckhof et al., 2009). Some preys may contribute more to the diet during daytime (e.g. *J. herdmani*), other more during night time (e.g. *Liocarcinus* spp. and *Brachyura* sp.), while some (e.g. *P.*

longicornis) are important throughout the day. Hence, the prey composition may change somewhat during the course of a day, but the prey availability remains constantly high. Therefore Atlantic cod behaviour is suggested not to be linked to specific prey species, but to the prey community as a whole.

Feeding behaviour is expected to reflect the optimal energy gain (Kerr, 1982). Atlantic cod can use both visual and chemical senses to localize prey (Brawn, 1969). However, during the daytime they have a bigger chance to encounter and locate prey (Løkkeborg and Fernö, 1999), making it energetically more profitable to forage during this period of the day.

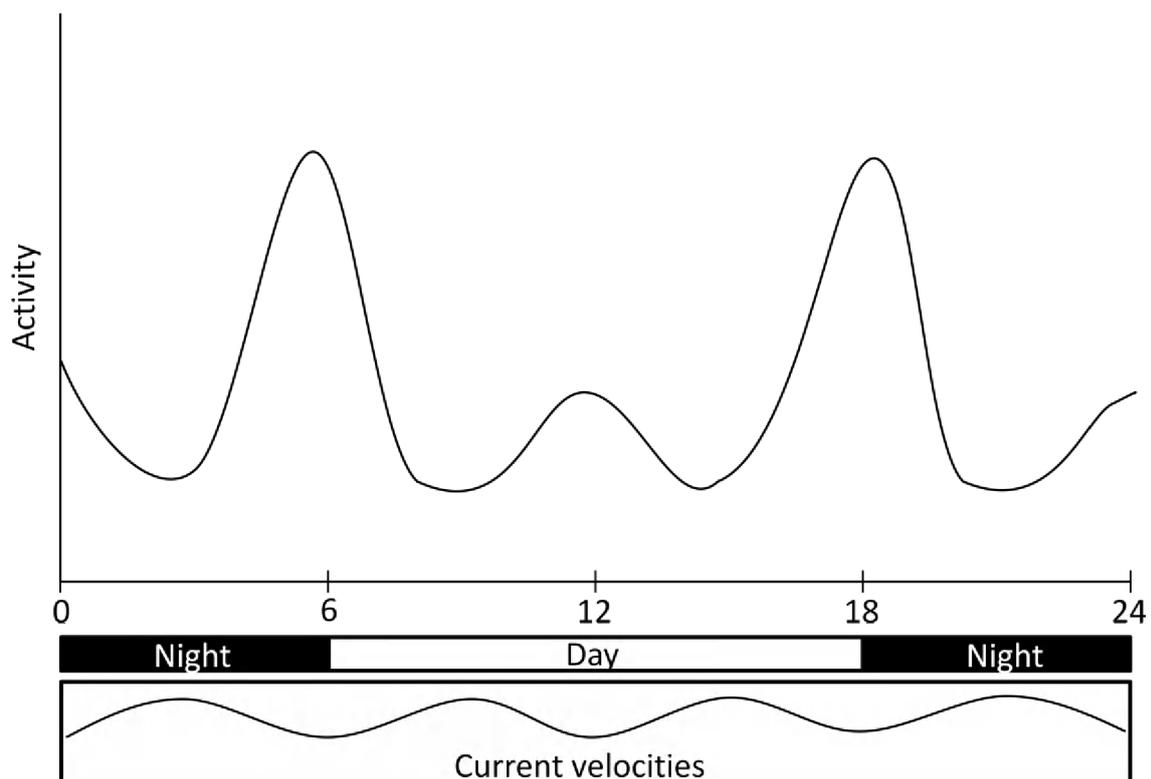


Figure 5. Conceptual representation of the diurnal activity patterns of Atlantic cod at the windmill artificial reefs in the Belgian part of the North Sea. Atlantic cod are most active during twilight periods, followed by a smaller activity peak during periods of low current velocity. This activity pattern is assumed to be caused by a trade-off between maximizing energy gain (foraging success) and minimizing predation probability.

Predation pressure

Based on the previous arguments Atlantic cod is expected to actively forage during daytime. However, crepuscular feeding behaviour was observed. Therefore, the risk of predation is probably influencing their feeding behaviour as well. Løkkeborg & Fernö (1999) investigated diel food search behaviour in Atlantic cod in a Norwegian fjord where the predation pressure was assumed to be negligible. The results indicated that Atlantic cod was more active during daytime, supporting our idea. Gregory & Anderson (1997) observed that 2-4 year old Atlantic cod were often associated with specific substrate features (such as rocks, crevices and holes) which represent potential cover against predators. At the WARs, the scour protection forms

a habitat with a high complexity. The stone mattress of boulders and rocks creates an ideal hiding place, with many holes and crevices. During scuba diving operated visual surveys at these WARs Reubens et al. (2011) occasionally observed Atlantic cod of 2 to 3 years old in these crevices.

The harbour porpoise, harbour seal, grey seal and white-beaked dolphin are four natural predators of Atlantic cod that occur in the BPNS (Haelters et al., 2011). The harbour porpoise is present year round and may reach seasonally high abundances (more than 1 ind./km²) (Haelters et al., 2012). Based on stranding and sighting information it has been shown that the numbers of this species have increased in recent years in the southern North Sea (Haelters and Camphuysen, 2009). Harbour porpoises feed on a wide variety of fish species, with Atlantic cod (and gadoid species in general) often as one of the main species in their diet (Santos and Pierce, 2003). Harbour porpoises are regularly present at offshore installations in the North Sea and an acoustic monitoring study in a Dutch offshore wind farm revealed that the activity of harbour porpoises was significantly higher inside the wind farm than in the reference areas. It is suggested that these structures may play an important role as porpoise feeding stations (Scheidat et al., 2011; Todd et al., 2009).

The harbour seal, grey seal and the white-beaked dolphin are also occasionally observed in Belgian waters (Haelters et al., 2011). The diet of both seals is linked to availability of prey species and Atlantic cod is often consumed (Hall et al., 1998; Hammond et al., 1994). Both harbour and grey seals have been observed within the offshore wind farms in the North Sea (personal observations, Tougaard et al., 2003). The white-beaked dolphin has become the most numerous cetacean after the harbour porpoise in the Southern North Sea (Jansen et al., 2010, www.waarnemingen.be). The dolphins are highly selective and mainly feed upon whiting and Atlantic cod. In addition, Atlantic cod present in stomachs of stranded white-beaked dolphins in the Netherlands, had an average length of 38 and 36 cm in adult and juvenile dolphins respectively (Jansen et al., 2010). The average length of Atlantic cod that occurred at the WARs in the BPNS is 36 cm, rendering them the ideal prey size for the dolphins.

Although no direct observations of predation events on Atlantic cod at the WARs are available it is assumed, based on the previously mentioned literature information, that the risk of predation influences the observed diel feeding behaviour of Atlantic cod. Foraging at low ambient light levels (i.e. at dusk and dawn) probably causes a trade-off between foraging success and reducing predation pressure.

Other benefits stimulating aggregation

Next, the present results indicated that Atlantic cod occurs in the vicinity of the WAR throughout the 24h of a day. High detection rates were observed for acoustically tagged fish during both day and night (Table 2). This indicates that they do not leave the area in-between feeding periods. There might be other benefits stimulating the aggregation behaviour at the WARs besides food. Shelter against currents and reduced predator pressure are suggested to influence this behaviour (Bohnsack and Sutherland, 1985; Wilhelmsson et al., 2006).

The FFT (Fig. 2) revealed a secondary 6 h periodicity peak in the detection data. This peak is probably related to the tidal regime of the studied area (Fig. 5). Tides are semi-diurnal in this region and the tidal currents can reach high velocities (varying between 0.2 to 0.6 m/s at neap tide and 0.3 to 0.9 m/s at spring tide). The swimming activity of Atlantic cod is known to decrease in periods of strong currents (Løkkeborg et al., 1989), which is probably related to energy optimization. Around the concrete turbines there is always one side that provides shelter against the currents and many hiding places are present between the rocks of the scour protection (personal observations). Atlantic cod may also maintain position by heading upstream at slow swimming performance (Løkkeborg et al., 1989, personal observations). The energetic cost associated with this sustainable swimming speed is very low for Atlantic cod (Soofiani and Priede, 1985).

Offshore wind power development – a broader context

In recent years offshore wind farms arose all across the North Sea (Arapogianni et al., 2013; Brabant et al., 2012) and member states are planning a further monumental development (Wilhelmsson and Malm, 2008). As a result thousands of wind turbines will be present in the North Sea in the near future. In the BPNS two wind farms are already (partially) operational: C-Power and Belwind. In the near future five more wind farms will be constructed. As a result more the 400 wind turbines will be present in the BPNS (Brabant et al., 2012; Rumes et al., 2011a; Rumes et al., 2011b). This creates a large potential for Atlantic cod populations in the Belgian part of the North Sea and beyond. As long as fisheries activities are banned inside the wind parks, the fish residing in this habitat are less vulnerable to fishing mortality; resulting in higher survival rates. From a management perspective, it is therefore essential to carefully monitor the fish populations present in offshore wind farms to broaden the knowledge on fish ecology at WARs. Thorough management restrictions should be implemented to allow fish populations to fully exploit the benefits from this protective habitat.

This study provided the first empirical data on the diel movements patterns of Atlantic cod at WARs in the BPNS (Fig. 5). They exhibited crepuscular movements related to feeding activity. We suggest that this crepuscular behaviour is related to the prey species community and to predation pressure. Preys are available throughout the day, but also predators may occur at the WARs. Therefore, foraging at low ambient light levels (i.e. at dusk and dawn) probably causes a trade-off between foraging success and reducing predation pressure.

Next, the results showed that Atlantic cod resided at the WAR in-between feeding periods. The integrated approach, combining acoustic telemetry with stomach content analysis and catch rate information, greatly contributed to the interpretation of the data. We therefore strongly encourage multidisciplinary approaches in future research to investigate fish ecology.

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Chapter 7

Energy profiling of demersal fish

Adapted from:

De Troch M, Reubens J, Heirman E, Degraer S, Vincx M, Submitted. Energy profiling of demersal fish: a case-study in wind farm artificial reefs. Marine Environmental Research¹.

ABSTRACT

The construction of wind farms introduce artificial hard substrates in sandy sediments. To test the reef effect on local fish that often aggregate around it, energy profiling and trophic markers were applied to study the feeding ecology of Atlantic cod and pouting and some of their potential prey in the Belgian part of the North Sea. The proximate composition (carbohydrates, proteins and lipids) differed significantly between liver and muscle tissue but not between fish species or between prey species. Atlantic cod (*Gadus morhua*, Gadidae) showed to consume more energy than pouting. The latter had a higher overall energy reserve and can theoretically survive twice as long on the available energy than cod. In autumn, both fish species could survive longer on their energy than in spring. Polyunsaturated fatty acids were found in high concentrations in fish liver. The prey species *Jassa* and *Pisidia* were both rich in EPA while *Jassa* had a higher DHA content than *Pisidia*. Energy profiling supported the statement that wind farm artificial reefs are suitable feeding ground for both fish species. Sufficient energy levels were recorded.

Key words: Atlantic cod, pouting, energy profiling, fatty acids, proteins

¹The contribution of Reubens J. to the manuscript is 40 %, De Troch M. did 60 % of the work. Practical work was done by Heirman E. (Master thesis EMBC, supervised by De Troch M and Reubens J.).

1. INTRODUCTION

Global concern on climate change together with decreasing non-renewable fossil fuel supplies has led to an increasing interest in generating electricity from renewable energy sources (Gill, 2005; Pelc and Fujita, 2002). Therefore, a massive expansion of offshore wind power is under preparation in North-western Europe, with some 10.000 offshore turbines planned to be constructed in the near future (Wilhelmsson et al., 2006). Constructing offshore wind turbines introduces artificial hard substrates in a region that is mainly characterized by sandy sediments. With the construction of wind farms in the sandy sediments of the Belgian part of the North Sea (BPNS) (currently 91 wind turbines, more than 200 to be constructed) a unique situation is created to investigate the effects of these artificial hard substrates. This change of habitat type (from sandy to hard substrate) is called the reef-effect and is considered as one of the most important changes of the marine environment (Kerckhof et al., 2010a). The construction of wind farms can also affect the marine environment through noise, electromagnetic fields and changes in hydrological conditions (Wilhelmsson et al., 2006).

In contrast to these expected negative effects, natural or man-made solid structures on the seabed are known to be effective in attracting and concentrating fishes (Bohnsack and Sutherland, 1985; Pickering and Whitmarsh, 1997; Reubens et al., 2013; Reubens et al., 2011; Wilhelmsson et al., 2006). Artificial reefs (AR), such as oil platforms, breakwaters, pontoons, shipwrecks and windmill foundations serve also as habitats for fishes and invertebrate assemblages (Wilhelmsson et al., 2006). Local fish aggregations in the BPNS (e.g. Atlantic cod (*Gadus morhua* L.) and pouting (*Trisopterus luscus* L.) were observed in the vicinity of the Belgian wind turbines (Reubens et al., 2011; Reubens et al., 2010). This supports the function of wind farms as AR as the same fish species are also attracted to shipwrecks studied in the BPNS (Zintzen et al., 2006).

Moreover, the irregular rough reef surfaces promote the settlement of sessile organisms allowing fouling communities to establish (Hixon and Brostoff, 1985; Kerckhof et al., 2010a). These communities are an important source of food for fishes and other organisms. Adding hard bottom habitat can thus turn a low productive environment into a dynamic, highly productive system providing direct shelter and food for many organisms (Stone et al., 1979).

In spite of the growing evidence of higher fish densities and biomasses at ARs compared to the surrounding areas (Wilhelmsson et al., 2006), it remains subject of debate whether the reefs actually generate new fish biomass or solely attract fish (Bohnsack, 1989; Bohnsack and Sutherland, 1985; Pickering and Whitmarsh, 1997). The attraction hypothesis is mainly based on behavioural preferences, whereas the production hypothesis assumes an actual increase of the carrying capacity of the system which will eventually lead to an increase in the abundance and

biomass of reef fishes (Bohnsack, 1989). Mechanisms that can contribute to the latter are additional food availability, higher feeding efficiency, shelter from predation and currents, and provision of recruitment habitat for settling organisms (Bohnsack, 1989; Randall, 1963; Reubens et al., In press-a; Stone et al., 1979). The relative importance of the attraction versus production hypotheses is likely to depend on the physical characteristics and the location of the reef (Bohnsack and Sutherland, 1985).

In order to evaluate the attraction versus the production hypothesis, it is necessary to analyse the feeding ecology of the fish in the AR. More specifically, not only trophic interactions should be studied but also whether the new habitat can support the necessary energy to maintain the increased population. Only in the latter case, the production hypothesis can be validated. Better knowledge on the potential of AR as feeding, breeding and nursery grounds for fish may generate insights for possible co-use of these specific areas by e.g. sustainable energy industry and fisheries (see e.g. Verhaeghe et al., 2011).

Recent studies on the stomach content analysis of Atlantic cod and pouting caught near the wind farms in the BPNS revealed that the tube building amphipod *Jassa herdmani* and the long-clawed porcelain crab *Pisidia longicornis* are the most important prey species (Reubens et al., 2011; Reubens et al., 2010). These epifaunal species were also the dominant hard substrate species present on the windmill foundations (Kerckhof et al., 2010a) suggesting that the fish come to feed on the epifaunal species on the pillars and profit from the larger erosion protection. However, stomach analyses can underestimate the importance of soft and highly digestible food items and overestimate that of recently consumed items (Graeve et al., 2001; Latyshev et al., 2004).

Far beyond the 'snapshot' level of resolution provided by stomach analysis, the use of trophic biomarkers and energy profiling allows to study the feeding ecology of consumers and to estimate the energy transfer from prey to consumer on the long-term (Iverson et al., 2004). In the present study, the proximate composition (proteins, lipids and carbohydrates) and the energy content (based on respiratory electron transport system) were estimated for two abundant and commercially relevant fish species (i.e. Atlantic cod and pouting) and some of their potential prey, sampled at an offshore wind farm in the BPNS. This functional approach will contribute to a better explanation of the occurrence and attraction of the target fish species to this specific site. Moreover, the obtained data on the energy levels, both in prey and consumer, will allow to draw conclusions on the contribution of AR in the energy flow between primary and secondary consumers. If this energy flow shows to be substantial, this would imply an important contribution to the production hypothesis for AR. So far, any information on the nutritional value of particular prey in the overall diet of Atlantic cod and pouting at the wind

farm is lacking. Moreover, the obtained net energy budgets will allow to estimate how long the consumers can survive on the energy gained in the AR.

In addition to the overall energy profiling, fatty acid (FA) profiling of prey and consumers was included as FA are known as important biomarkers (so-called trophic markers). By means of FA profiling, we aim to trace any directional assimilation of a particular FA in order to estimate in every detail what a particular prey contributes to the FA pool of the consumer. Here, we opted to analyse total FA, including structural FA used for growth and FA stored as reserve. Special attention was given to the presence of polyunsaturated FA (PUFA) as important label for dietary quality (Dalsgaard et al., 2003), also for human consumption.

The specific objectives of this study are (1) to quantify proximate composition and energy content of Atlantic cod and pouting and their main prey species and (2) to identify the contribution of dominant prey species (*J. herdmani* and *P. longicornis*) to the diet and energy requirements of both fish species. Ultimately, in combination with data on density and productivity of the prey species, it should be possible in the future to estimate whether the fish species obtain sufficient energy from the food sources available at the AR in wind farms to sustain their basal metabolism or growth.

2. MATERIAL & METHODS

2.1 Field sampling

Samples were collected in the BPNS at the C-Power wind farm (51°33'N – 2°56'E) on the Thorntonbank, a natural sandbank situated 27 km off the Belgian coast. The windmill foundations surveyed in this study are surrounded by a scour protection layer to prevent the erosion of the backfill sediment around the foundations. This protection layer consists of two layers: a filter layer of about 55.5 m diameter for which crushed gravel with a diameter of 10 to 88 mm was used; and an armour layer with a diameter of about 51 m consisting of quarried rock (Brabant and Jacques, 2010; Reubens et al., 2011). The scour protection layer together with the foundation forms the AR.

In the period of October - November 2011, several sampling campaigns were organized at the wind farm with the research vessel 'Zeeleeuw'. Atlantic cod and pouting were collected by line fishing (hook type: Arca, size n° 4) with the lugworm *Arenicola marina*, fresh or frozen, as bait. Angling was performed close to the turbine (1-10 m distance) just above the armour layer to make sure that the fish caught were associated at that moment with the AR. Muscle and liver tissue was collected from 10 individuals and frozen at -80°C until further analysis. Fish length ranged between 39-46 cm and 20-22 cm respectively for Atlantic cod and pouting. The obtained data were compared with a similar yet smaller dataset collected in spring (February-March

2011) consisting of 5 and 6 individuals of Atlantic cod and pouting, respectively. Fish lengths were 21-45 cm (Atlantic cod) and 19-22 cm (pouting).

To collect the prey species, small rocks were taken by divers from the armour layer of the windmill pillars. The prey species present on the rocks were identified and sorted on board, and frozen at -80°C until further biochemical analysis in the laboratory. Triplicate samples of the prey species *Jassa herdmani* and *Pisidia longicornis* were available. The prey species are further referred to by the genus names. *Jassa* is a tube-dwelling amphipod, constructing tubes that can form organic mats (Kerckhof et al., 2010a). These mats were analysed separately from *Jassa* after picking out the amphipods.

2.2 Biochemical analyses

2.2.1 Energy availability (E_a)

The proximate composition (proteins, carbohydrates and lipids) was determined to quantify the total available energy. Samples of liver and muscle tissue of the fish species and whole organisms of the prey species were subjected to a cellular energy allocation (CEA) protocol. The original CEA protocol was developed by De Coen and Janssen (1997) to measure the available and consumed energy of *Daphnia magna*, and was later used for e.g. mysid shrimp (Verslycke et al., 2004) and zebra mussel (Smolders et al., 2004). The original CEA protocol, was adapted by performing two additional sonication steps to obtain a more complete homogenisation of the samples: frozen samples were crushed with a pestle in 1 ml homogenisation buffer. Thereafter, the crude homogenate was subjected to 0 to 4 rounds of sonication, using a Sonics Vibra-cell VCX-500 Ultrasonic Processor (pulse: 2-1 sec; amplitude: 25%; time: 1 min). As stable values of protein content were measured after two extra sonication rounds, this adaptation of the protocol was applied for all further analyses.

Carbohydrates

The protocol to determine carbohydrate content was actually developed to measure both proteins and carbohydrates in a single sample (De Coen and Janssen, 1997). Therefore, frozen samples were weighted and homogenising in 1 ml milliQ water using a micro pestle and subsequently proteins (see further) were precipitated by addition of 500 µl of 15% Trichloroacetic acid (TCA).

After centrifugation (10 min at 2000 g), the supernatant was collected. The pellet was resuspended in 200 µl of 5% TCA, vortexed and centrifuged again (10 min at 2000 g). The collected supernatant was added to the former one and vortexed, after which 250 µl of 5% phenol and 1 ml of concentrated sulphuric acid were added. Three replicates of each 300 µl

were added to a 96-multiwell plate. After 15 minutes of incubation in the dark, the absorbance was measured at 492 nm using a Victor Multilabel Reader (PerkinElmer). A standard curve based on a 0.5% glucose solution was used to calculate the carbohydrate concentration.

Proteins

To determine the protein content of the samples the Bradford method was used. This is a colorimetric method which involves the binding of the dye Coomassie Brilliant Blue G to a protein (Bradford, 1976). Samples (± 0.5 g) were homogenized in 1 ml Tris (0.05 mol/l, pH 6.8). Complete homogenisation was achieved after two rounds of sonication (pulse: 2-1 sec; amplitude: 25%; time: 1 min). Hereafter, the samples were centrifuged for 20 minutes at 6800 g in a microcentrifuge (Eppendorf Centrifuge 5810R). From each sample, 3 replicates of 25 μ l were placed in a 96 multiwell plate. Next, 250 μ l of Bradford reagent (containing the dye Coomassie Brilliant BlueG) was added to each well and, after 15 minutes of incubation in the dark, the optical density was measured at 595 nm (De Coen and Janssen, 1997) using a Victor Multilabel Reader (PerkinElmer) with Bovine Serum Albumin (BSA) used as standard. The protein concentration in the tissue (X_s) was obtained from a regression between the standard concentration (mg/ml) and the measured absorbance and further standard as protein concentration per weight of tissue (mg/mg) following the equation $X_s = \frac{x \cdot V_{well}}{DF \cdot m}$ with V_{well} : volume of sample added to the well (ml); DF: dilution factor (volume applied in the well vs. total volume available after centrifugation), m: wet weight of the sample (mg).

Lipids

Total lipids were extracted following the method of Bligh and Dyer (1959). The samples (muscle, liver and prey) were homogenized in 450 μ l milliQ water followed by two rounds of sonication (pulse: 02-01 sec; amplitude: 25%; time: 01 min). Then 500 μ l of methanol and 500 μ l of chloroform were added. After centrifugation (10 min, 1800 g), the top phase was removed and 500 μ l of H₂SO₄ was added to the dried lipid extract and charred for 15 min at 200°C. Data on the lipid content in *Pisidia* is not reported due to shortage of material.

The different E_a fractions for the liver and muscle tissues of both fishes and for the whole organisms of the prey species were transformed into energetic equivalents by using the enthalpy of combustion: 17.5 J/mg carbohydrates; 24 J/mg proteins; and 39.5 J/mg lipids (Gnaiger, 1983). The total available energy was calculated as the sum of these different fractions.

Although water does not contribute to the available energy, the water content was estimated for all samples in order to complete the proximate composition. Samples were dried at 60°C until constant weight. This temperature was chosen to avoid loss of volatile lipids at higher temperatures (Hyslop, 1980) and thus yielding an overestimation of the water content. Dry weight was subtracted from the wet weight to obtain the water content.

2.2.2 Energy consumption (E_c)

To estimate the energy consumption (E_c) by the fish and prey species, the activity of the respiratory electron transport system (ETS) was calculated. Since the ETS controls oxygen consumption, the activity of this ETS provides an estimate of the potential respiration rate and thus energy consumption (Packard, 1968). INT (p-iodonitrotetrazolium violet) replaces O_2 as electron acceptor in the cell's mitochondria, where the ETS is found. Reduction of this INT will result in the formation of formazan, which has a red colour. Two μmol of INT corresponds to 1 μmol of O_2 (De Coen and Janssen, 1997).

After measuring of wet weight, samples were homogenized in 400 μl of homogenate buffer, containing 0.01 M PO_4 buffer pH 8.5 (476 ml 0.01 M Na_2HPO_4 + 24 ml 0.01 M K_2HPO_4), 0.05 M Tris, 75 $\mu\text{mol/L}$ $MgSO_4$, 1.5 mg/ml PVP (polyvinylpyrrolidone) and 0.2% Triton X-100. After centrifugation, three replicates of 60 μl of supernatant were added to the multiwell plate. To each replica 180 μl of buffered substrate solution was added. This buffered substrate solution containing 0.01 M PO_4 was added, to which 0.05 M Tris and 0.2 % Triton X-100 were added, as well as 1.7 mM NADH and 0.25 mM NADPH which are ETS stimulators. Immediately after adding 60 μl of INT to each well, the kinetic reaction needed to be measured every 7 seconds at 490 nm.

The maximal rate per minute (V_{max}) was calculated to use in the following formula:

$$\text{Mol } O_2 \text{ min}^{-1} \text{ m}^{-1} = \frac{V_{\text{max}} \cdot V_{\text{well}}}{2 \cdot \varepsilon \cdot l \cdot DF \cdot 10^6 \cdot m}$$

For one hour: $\text{mol } O_2 \text{ hour}^{-1} = \text{mol } O_2 \text{ min}^{-1} \times 60$

Energy value: $\text{kJ hour}^{-1} = \text{mol } O_2 \text{ hour}^{-1} \times 484 \text{ kJ} \times \text{mol}^{-1} O_2$

V_{max} : $\Delta\text{Abs}/\Delta\text{time}$; V_{well} : total volume in well (300 μl); DF: dilution factor (60/400 = 60 μl is used for measurement out of 400 μl homogenate available); 2: 2 mol formazan per mol O_2 .

The formula is based on the formula of Lambert-Beer: $A = \varepsilon \times l \times c$ with A: absorbance; ε : extinction coefficient, l: optical length and c: concentration. In this protocol: ε for INT-formazan = $15900 \times (\text{mol/l})^{-1} \times \text{cm}^{-1}$ and l: 0.7795 cm, optical length of multiwell filled with 300 μl .

The amount of oxygen that was consumed per sample (estimated from the ETS data) was transformed into energetic equivalents by using the specific oxyenthalpic equivalents for an average lipid, protein, and carbohydrate mixture of 484 kJ/mol O₂ (Gnaiger, 1983).

2.2.3 Cellular Energy Allocation (CEA)

The available (E_a) and consumed (E_c) energy can be integrated into an overall net-energy budget (De Coen and Janssen, 2003). This cellular energy allocation (CEA) can be calculated as E_a/E_c and reflects the energy status of an organism at the cellular level. To estimate the CEA of the whole fishes, the calculated CEA values for liver and muscle samples were used. Based on the liver weight (preserved on 8 % formaldehyde-seawater solution) and total weight of 350 individuals of cod (Reubens, personal database) it was estimated that the preserved liver weight constituted about 2.5 % of the total weight. Muscle mass represents about 55 % of the total weight in cod (Lambert and Dutil, 1997a). For pouting, the preserved liver constituted about 2.98 % of the total body weight (n=120 individuals) (Reubens, personal database). The relative muscle mass in pouting was not found in literature, so it was decided to use the same number as for cod, i.e. 55 %. As most of the energy in the fishes is present as proteins and lipids in the muscle and the liver, the following equations were used: total cellular energy allocation (CEA_T) = (2.5 % CEA_{liver}) + (55 % CEA_{muscle}) for cod and (CEA_T) = (2.98 % CEA_{liver}) + (55 % CEA_{muscle}) for pouting.

2.3 Fatty acid analysis

Fatty acid profiles of prey species and consumers (fish) were analyzed to allow both qualitative and quantitative analyses of the diet of the predators (Budge et al., 2006), and thus showed the relative importance of different prey species.

Hydrolysis of total lipid extracts of the prey samples, muscle and liver tissue samples of the fish species) and methylation to fatty acid methyl esters (FAME) was achieved by a modified one-step derivatisation method after Abdulkadir and Tsuchiya (Abdulkadir and Tsuchiya, 2008; De Troch et al., 2012). The boron trifluoride-methanol reagent was replaced by a 2.5 % H₂SO₄-methanol solution since BF₃-methanol can cause artefacts or loss of polyunsaturated fatty acids (PUFAs) (Eder, 1995). The fatty acid Methylnonadecanoate C19:0 (Fluka 74208) was added as an internal standard for the quantification. Samples were centrifuged (Eppendorf Centrifuge 5810R) and vacuum dried (Rapid Vap LABCONCO). The FAME thus obtained were analysed using a Hewlett Packard 6890N GC coupled to a mass spectrometer (HP 5973). The samples were run in splitless mode (1 µl injected), except for the livers which were run in split mode (1/10 µl injected) because there was an overload of material. This was done with a 10 µL injector per

run, at an injector temperature of 250 °C, using a HP88 column (60 m × 25 mm i.d., Df = 0.20; Agilent J & W; Agilent Co., USA) with He flow rate of 1.3 ml min⁻¹. The oven temperature was programmed at 50 °C for 2 min, followed by a ramp at 25 °C min⁻¹ to 175 °C and then a final ramp at 2 °C min⁻¹ to 230 °C with a 4 min hold. The FAME were identified by comparison with the retention times and mass spectra of authentic standards and mass spectral libraries (WILEY, NITS05), and analysed with the software MSD ChemStation (Agilent Technologies).

Quantification of individual FAME was accomplished by the use of external standards (Supelco™ 37 Component FAME Mix, Supelco # 47885, Sigma-Aldrich Inc., USA). The quantification of each individual FAME was obtained by linear regression of the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 µg ml⁻¹).

2.4 Statistical analysis

Differences in proximate composition (proteins, carbohydrates and lipids) and energy content between (1) both fish species and different tissues (liver vs. muscles); (2) among prey species and (3) between the fish and prey species were tested by means of two-way analyses of variance (ANOVA) for (1) and one-way ANOVA for (2) and (3). The software Statistica 6.0 software (StatSoft Inc., 2001) was used. Prior to all ANOVAs, the Cochran's C-test was used to check the assumption of homoscedasticity. Normality was tested using Shapiro-Wilk's test.

Multivariate analysis of FA compositions was conducted with a non-metric multidimensional scaling method (MDS) based on Bray-Curtis similarity using Primer 5 software (Clarke and Gorley, 2006). Subsequently, a one-way analysis of similarities (ANOSIM) was used to test for significant differences between the groups based on their origin (i.e. liver, muscle, individual prey species). Finally, percentages of similarity (SIMPER) were calculated with square-root transformed, absolute FA concentrations to determine the main FA contributing to any differences. The concentration of these indicative FA were represented in bubble plots to clarify these differences.

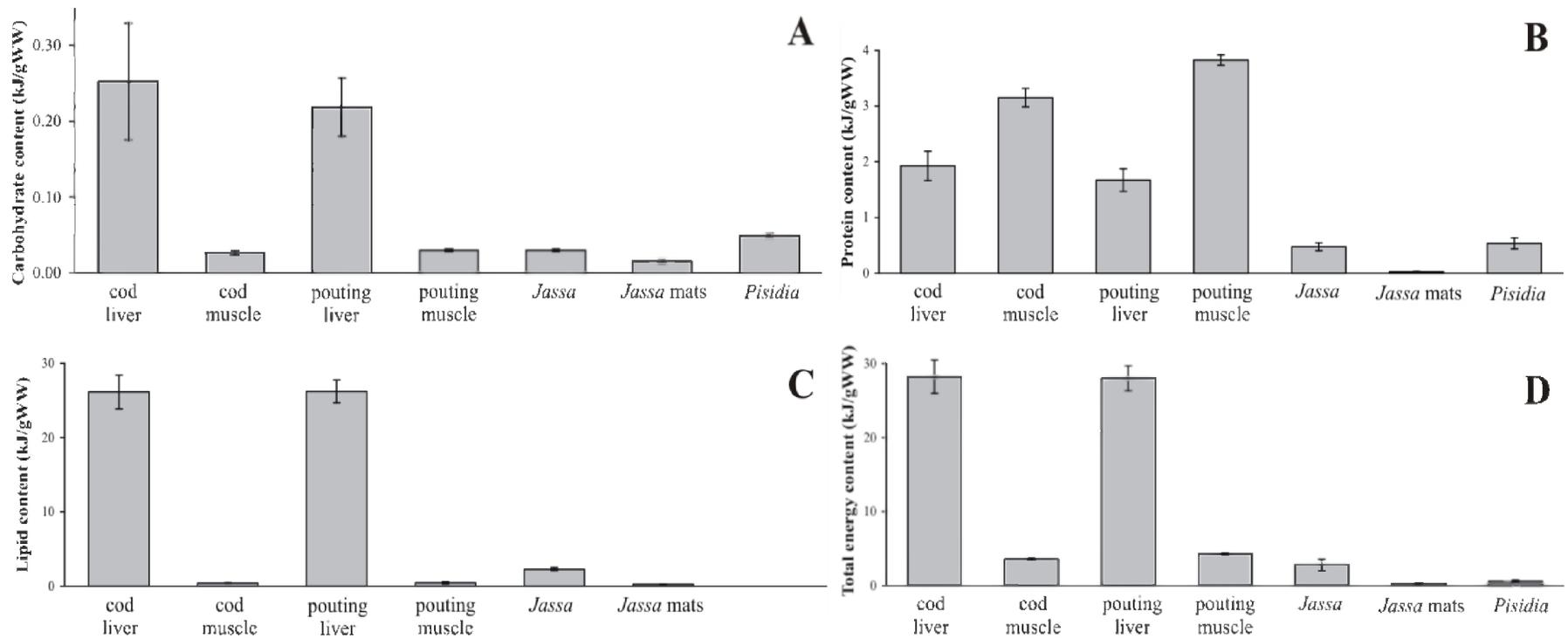


Figure 1. Average (\pm SE) energy content (protein, lipid, carbohydrate and total) in kJ/gWW for liver and muscle tissue of cod and pouting, and for *Jassa*, *Jassa mats* and *Pisidia*.

3. RESULTS

3.1 Energy availability (E_a)

In general, the carbohydrate content (Fig. 1a) in all samples was low, i.e. below 0.25 kJ/gWW and not reaching more than 1 % WW. Liver tissue of both cod and pouting (resp. 0.25 ± 0.08 and 0.22 ± 0.04 kJ/gWW) contained significantly more carbohydrates than the corresponding muscle tissue (both 0.03 ± 0.00 kJ/gWW)(two-way ANOVA, $p < 0.01$ for tissue, $p = 0.8$ for species). The reverse trend was found for protein concentration (Fig. 1b) being significantly higher in the muscle samples (resp. 3.15 ± 0.16 and 3.83 ± 0.09 kJ/gWW) than in the liver samples (resp. 1.93 ± 0.26 and 1.67 ± 0.20 kJ/gWW) for both cod and pouting (two-way ANOVA, $p < 0.001$ for tissue, $p = 0.3$ for species). The lipid content (Fig. 1c) was highest in the liver samples and very similar for cod and pouting (resp. 26.13 ± 2.27 and 26.21 ± 1.55 kJ/gWW) although with a high variance between the replicates. In the muscle samples of cod and pouting the lipid content was very low (resp. 0.40 ± 0.03 and 0.44 ± 0.14 kJ/gWW).

The prey species did not show any major differences in terms of proximate composition. The carbohydrate content was low in both prey species (Fig. 1a), reaching the highest levels in *Pisidia* (0.05 ± 0.003 kJ/gWW), followed by *Jassa* (0.03 ± 0.002 kJ/gWW). The protein content (Fig. 1b) of *Jassa* and *Pisidia* was resp. 0.48 ± 0.07 and 0.54 ± 0.10 kJ/gWW. The lipid level was only determined for *Jassa* (2.28 ± 0.215 kJ/gWW), due to lack of sufficient *Pisidia* specimens. The *Jassa* mats were very low in the measured energy components (0.02 ± 0.003 kJ/gWW carbohydrates, 0.03 ± 0.006 kJ/gWW proteins and 0.25 ± 0.03 kJ/gWW lipids).

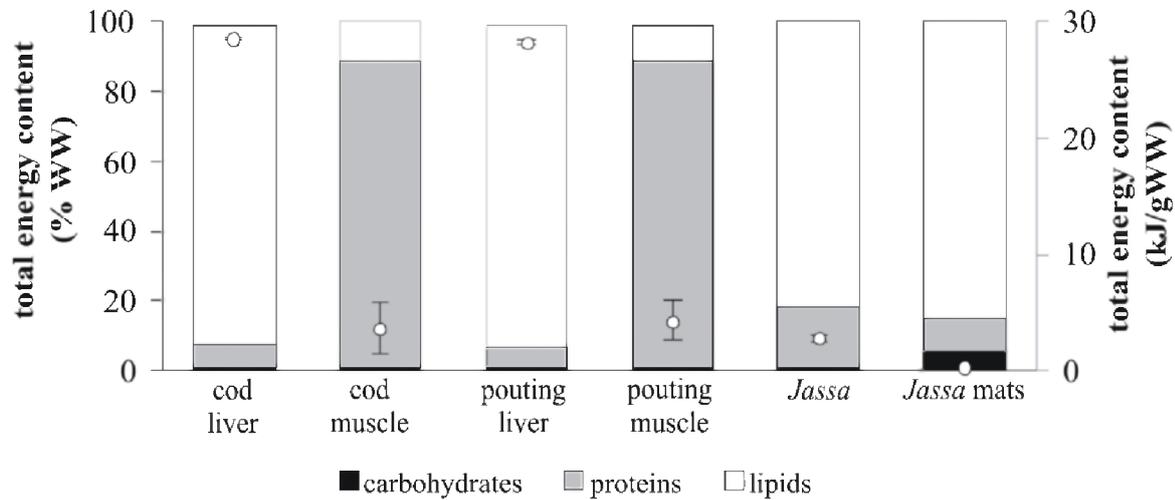
In cod and pouting the amount of water was higher in the muscle ($80 \pm 1.1\%$ WW for both fishes) than in the liver (resp. $56 \pm 17.5\%$ WW and $75 \pm 6.6\%$ WW). For the prey species, the water content was higher in *Jassa* (79% WW) than in *Pisidia* (63% WW). Water content was not determined for the *Jassa* mats.

The contribution of different energy components to the total energy reserves (total E_a) showed some clear differences (Fig. 2). The energy levels in the muscle samples were mainly determined by the protein levels, reaching up to 88.0 % of total muscle energy content in cod and 88.4 % in pouting. Further, the lipid content was resp. 11.3 % and 10.2 % of total energy content in muscles of cod and pouting. The muscle carbohydrate level reached only 0.8% and 0.7% for cod and pouting, respectively.

In the liver, lipids contributed 91.5% to E_a of cod and 92.6% for pouting. Proteins constituted respectively 6.7% and 5.9% of the total liver energy levels in cod and pouting. The amount of carbohydrates in the liver was relatively low i.e. only 0.9% and 0.8% of the total liver energy for cod and pouting, respectively.

The procentual allocation of the E_a in prey species could only be determined for *Jassa* and *Jassa* mats as there was insufficient material to determine lipid content in *Pisidia*. Lipids constituted 81.8 % of total energy in *Jassa*, whereas proteins and carbohydrates only

contributed resp. 17.1% and 1.1% to the total energy reserves. In the *Jassa* mats, 85.0% of total energy reserves were found to be lipids, 9.5% were proteins and the remaining 5.4% were carbohydrates.



2. Different fractions of total energy content in %WW (bars, left y-axis) and absolute total energy content in kJ/gWW (dots, average \pm SE, right y-axis) for liver and muscle tissue of cod and pouting, and for *Jassa*, *Jassa* mats and *Pisidia*.

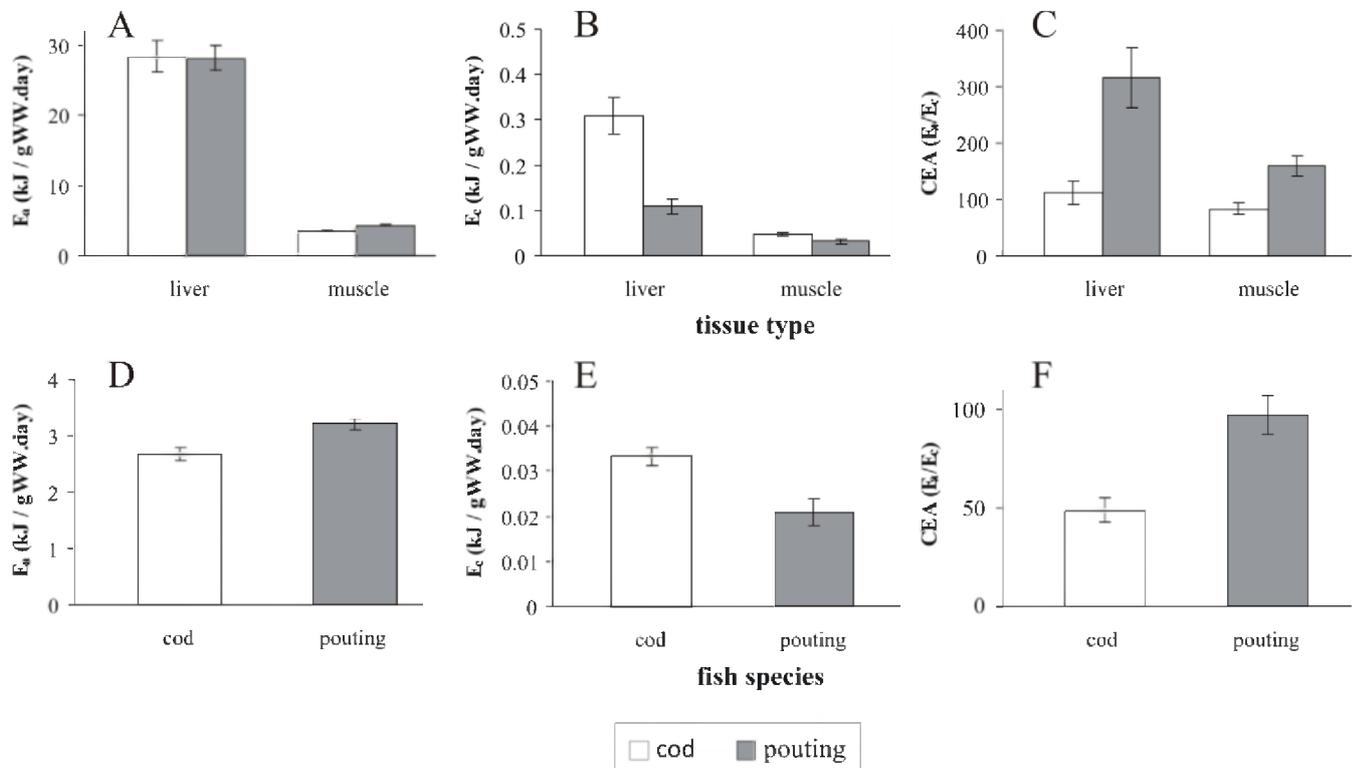


Figure 3. Average (\pm SE) available energy (E_a) (A, D), energy consumption (E_c) (B, E) and Cellular Energy Allocation (CEA) as E_a/E_c (C, F) per tissue (top figures) and per fish species (bottom figures)

3.2 Energy consumption (E_c)

The consumption of energy (E_c) was estimated for liver and muscle tissue separately (Fig. 3b). For both fish species, more energy was used in the liver tissue than in the muscles (Fig. 3b). The energy consumption rate was higher for cod (0.31 ± 0.04 kJ/gWW.day for the liver, 0.047 ± 0.003 kJ/gWW.day in the muscles) than for pouting (0.11 ± 0.02 kJ/gWW.day for the liver, 0.032 ± 0.005 kJ/gWW.day in the muscles). The overall energy consumed (E_c , Fig. 3e) calculated from these tissue-specific values showed the same pattern: 0.033 ± 0.002 kJ/gWW.day for cod vs. 0.02 ± 0.003 kJ/gWW.day for pouting.

3.3 Cellular Energy Allocation (CEA)

The cellular energy allocation (CEA) (Fig. 3 c,f) was calculated for both fish species and tissue types based on the available energy (E_a) (Fig. 3a, d) and the consumed energy (E_c) (Fig. 3b, e).

The overall energy reserves (Fig. 3f) are significantly higher in pouting (96.7 ± 9.9) than in cod (48.7 ± 6.3) (one-way ANOVA, $p < 0.001$). This implies that the former can survive twice as long on the available energy than the latter. The same pattern was found for liver and muscle of both species with the highest CEA values for the liver tissue (Fig. 3c). The low CEA of cod is clearly linked to its higher energy consumption (E_c , Fig. 3b, e) in spite of its high levels of E_a (Fig. 3a, d). Please keep in mind that the relative muscle mass of pouting is based on the one of Atlantic cod. This assumption may influence the CEA of pouting.

Autumn versus spring

The obtained values for energy available from the autumn samples (see before) were considerably higher than the ones found for a similar dataset obtained from fish in spring (table 1). The energy consumption of cod was similar between both seasons, but not for pouting. Especially the E_c values of the liver were considerably lower in autumn than in spring. All together, this led to a much higher cellular energy allocation ($CEA = E_a/E_c$), i.e. a fish could survive much longer on its energy, in autumn than in spring.

3.4 Fatty acid profiles

The non-metric MDS applied to the FA profiles (absolute concentrations) of both fish species, both tissue types (liver, muscle) and their potential food sources (Fig. 4a) revealed a clear separation between (1) the two tissue types of the fish and (2) between the food sources (2D stress: 0.04; overall ANOSIM, global R: 0.959, $p = 0.001$). It is worth mentioning that there is no clear difference between both fish species as their tissue samples plotted together (Fig. 4a).

Table 1. Average (\pm SD) energy content (protein, lipid, carbohydrate and total) in kJ/gWW for liver and muscle tissue of Atlantic cod and pouting, and for *Jassa*, *Jassa* mats and *Pisidia* in two seasons. Corresponding available (E_a) and consumed (E_c) energy are reported. Cellular energy allocation (CEA) is calculated as E_a/E_c .

	Cod Liver	Cod muscle	Pouting liver	Pouting muscle	<i>Jassa</i>	<i>Jassa</i> mats	<i>Pisidia</i>
AUTUMN							
Proteins	1.93 \pm 0.83	3.15 \pm 0.52	1.67 \pm 0.64	3.83 \pm 0.29	0.48 \pm 0.12	0.03 \pm 0.01	0.54 \pm 0.18
Carbohydrates	0.25 \pm 0.41	0.03 \pm 0.01	0.22 \pm 0.21	0.03 \pm 0.00	0.03 \pm 0.00	0.02 \pm 0.01	0.05 \pm 0.01
Lipids	26.13 \pm 7.17	0.40 \pm 0.13	26.21 \pm 4.89	0.44 \pm 0.44	2.28 \pm 0.37	0.25 \pm 0.05	n.d
Total E_a	28.31 \pm 7.11	3.58 \pm 0.54	28.10 \pm 5.38	4.30 \pm 0.47	2.781 \pm 0.26	0.29 \pm 0.03	0.59 \pm 0.10
Total E_c	0.31 \pm 0.13	0.05 \pm 0.01	0.11 \pm 0.05	0.03 \pm 0.02	0.01 \pm 0.00	0.00 \pm 0.00	0.02 \pm 0.00
E_a/E_c	112.44 \pm 66.87	83.47 \pm 36.5	315.54 \pm 166.08	158.64 \pm 52.27	570.99 \pm 245.09	116.58 \pm 56.40	29.59 \pm 0.10
SPRING							
Proteins	2.58 \pm 0.79	3.97 \pm 0.78	3.16 \pm 0.69	4.67 \pm 0.66	0.80	0.02	0.86
Carbohydrates	0.07 \pm 0.04	0.00 \pm 0.00	0.06 \pm 0.02	0.00 \pm 0.33	0.08	0.01	0.06
Lipids	11.29 \pm 6.49	0.15 \pm 0.14	4.12 \pm 6.44	0.29 \pm 0.33	0.68	0.05	0.77
Total E_a	13.93 \pm 5.82	4.13 \pm 0.67	7.34 \pm 6.07	4.96 \pm 0.68	1.56	0.08	1.69
Total E_c	0.33 \pm 0.15	0.04 \pm 0.01	0.55 \pm 0.21	0.05 \pm 0.01	0.06	0.00	0.06
E_a/E_c	56.07 \pm 38.43	111.62 \pm 42.85	19.68 \pm 25.96	95.04 \pm 22.83	24.04	-	28.16

Based on the absolute FA concentrations, there was a high similarity within each group of tissue (SIMPER, muscle: 89.9%, liver: 88.7%) and within each food source (SIMPER, *Jassa*: 94.8%, *Jassa* mats: 95.6%, *Pisidia*: 94.6%). The high level of similarity within the replicates of the tissue types was attributed to the FA docosahexaenoic acid or DHA (23.5% for muscle and 18.8% for liver), eicosapentaenoic acid or EPA (13.1% for muscle and 13.2% for liver) and 16:0 (14.9% for muscle and 11.2% for liver). The same FA explained most of the similarity within the *Jassa* (SIMPER contribution%: EPA: 19.7%, DHA: 17.7%, 16:0: 12.6%) and the *Jassa* mats samples (SIMPER, EPA: 17.0%, DHA: 16.1%, 16:0: 12.1%). The *Pisidia* samples were mainly characterised by EPA (18.4%), 16:1 ω 7 (13.8%), 16:0 (11.6%) and DHA (11.0%). As several FA were found in both fish tissue types, there was only 17% of dissimilarity between liver and muscle samples and DHA, 16:1 ω 7 and 18:1 ω 9t contributed most to this dissimilarity (SIMPER).

To eliminate the effect of different lipid concentrations between the two types of tissue, an additional MDS (2D stress: 0.08) on the relative FA concentrations was included (Fig. 4b). In this MDS, both fish tissues were separated from each other, but not as strong as for the absolute FA concentrations. *Pisidia* was found to plot separately from the other samples. There was a higher similarity between *Jassa* and the corresponding mats than what was found in the MDS on the absolute FA concentrations. SIMPER analysis revealed that the same FA as for the absolute concentrations contributed most to the similarities and dissimilarities within and between groups.

Based on the SIMPER results of the absolute FA concentrations (see before), the FA that contributed most to dissimilarity between groups were plotted (Fig. 5) and used as indicative biomarkers. The higher concentration of FA in the liver samples, as shown for 16:0 (Fig. 5a), is the general pattern that was found for other saturated FA (17:0, 18:0, 20:0; not shown). For the polyunsaturated FA (PUFA) ARA, EPA and DHA (Figs. 5 f-h) the highest concentration was also measured in the liver tissue. In all cases, the potential prey species showed much lower concentrations, except for EPA with considerable concentrations in *Jassa* and *Pisidia*. It is worth noticing that high concentrations of 14:1 ω 5 were detected in *Pisidia* (average concentration of $256.7 \pm 73.7 \mu\text{g/g DW}$) while it is absent in muscle tissue and only aliquots were found in the liver tissue (pouting: $103.1 \pm 59.5 \mu\text{g/gDW}$, cod: $34.0 \pm 19.6 \mu\text{g/gDW}$). The overall figure for 16:1 ω 7 is biased by the high concentration in one replicate of liver tissue (C l10) (fig. 5c) while SIMPER indicated this FA as contributing 13.8% to the similarity between the *Pisidia* replicates. There was indeed significantly higher concentration of 16:1 ω 7 in *Pisidia* ($11005.7 \pm 1878.5 \mu\text{g/gDW}$) than in *Jassa* ($6731.9 \pm 398.3 \mu\text{g/gDW}$) and the *Jassa* mats ($1336.5 \pm 50.2 \mu\text{g/gDW}$) (one-way ANOVA, $p < 0.01$).

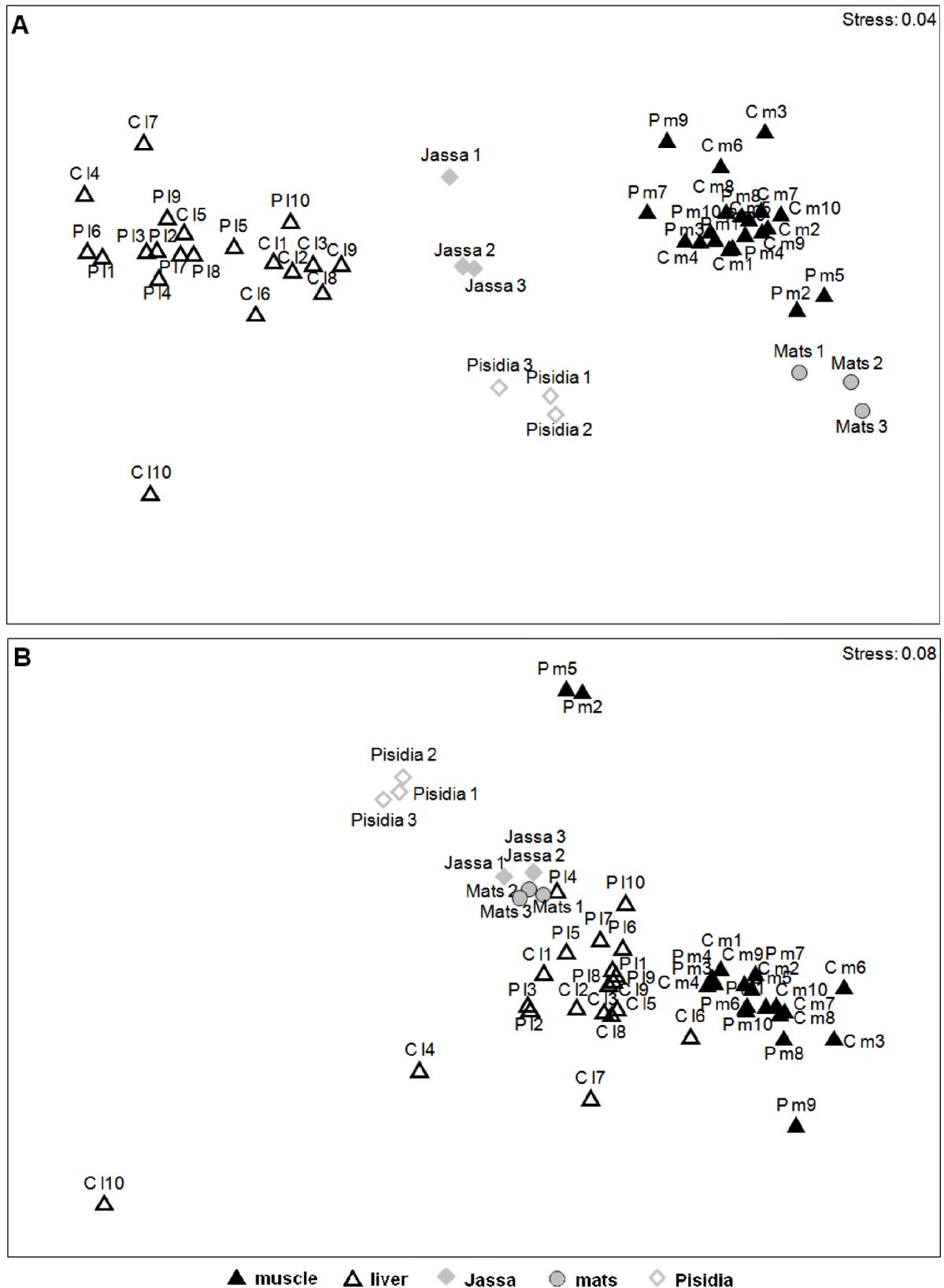


Figure 4. Non-metric multidimensional scaling (Bray-Curtis similarity) on (A) square-root transformed, absolute FA concentrations and (B) Arcsin transformed, relative FA concentrations for the fish tissues (l: liver, m: muscle) of both fish species (C: cod, P: Pouting) and prey items (*Jassa*, *Pisidia* and *Jassa* mats). Replicates indicated by numbers.

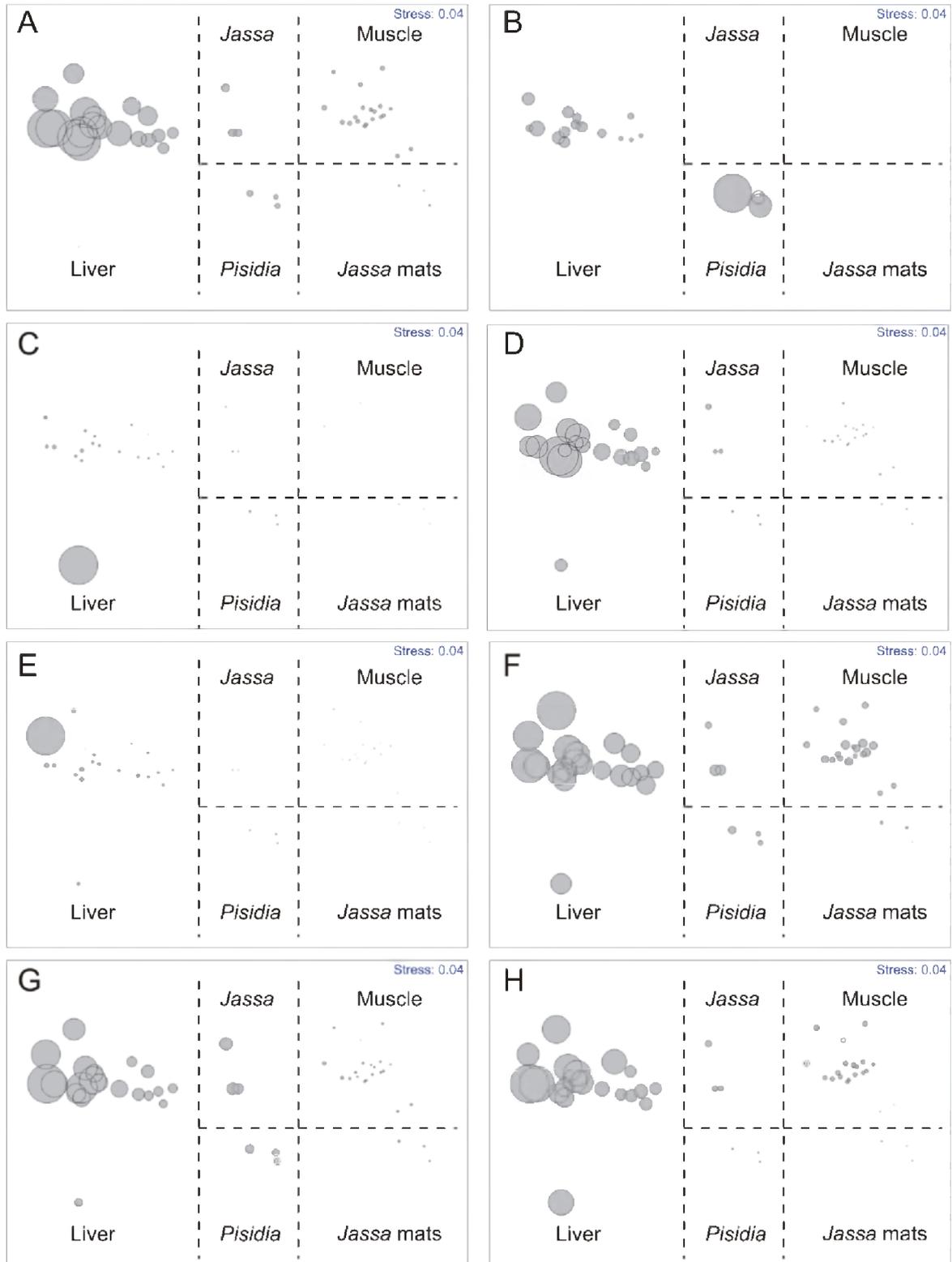


Figure 5. Bubble plots of indicative FA: (A) 16:0, (B) 14:1 ω 5, (C) 16:1 ω 7, (D) 18:1 ω 9t, (E) 18:1 ω 9c, (F) ARA, (G) EPA and (H) DHA, based on non-metric multidimensional scaling MDS (Bray-Curtis similarity) on square-root transformed, absolute FA concentrations for the fish tissues (liver, muscle) of both fish species and prey items (*Jassa*, *Pisidia* and *Jassa mats*) and SIMPER results. Detailed sample names as in Figure 4.

4. DISCUSSION

The energy profiling in the present study allowed to link the energy allocation of cod and pouting to the energy available in potential prey species. The adapted CEA protocol yielded energy levels within the reported ranges in previous studies (e.g. Eliassen and Vahl, 1982; Merayo, 1996b).

As in other gadoids, the muscles of cod and pouting turned out to be the main protein depots while lipid reserves are mainly stored in the liver (Lambert and Dutil, 1997a). In contrast, the muscles contained a very low lipid content (<1% of the muscle wet weight). This is a logical outcome as both fish species are considered as lean 'non-fatty' fishes (Lambert & Dutil, 1994). In gadoids, the liver is the major storage depot, where lipids form the main source of fuel reserve (Merayo, 1996b). Consequently, as the lipid content in liver was comparable for both fish species, a similar overall energy content can be expected.

Moreover, the lipid storage in the liver was found to be considerably higher in autumn than in spring, resulting into higher E_c and CEA. These results are consistent with the ecological behaviour of Atlantic cod and pouting. Both species are known for their seasonal patterns in growth and condition influenced by periods of energy accumulation and depletion due to interaction between feeding, maturation, reproduction and migration (Alonso-Fernández et al., 2008; Lambert and Dutil, 1997a; Mello and Rose, 2005a). Atlantic cod and pouting liver condition follow a seasonal cycle, enlarging when food availability is high during summer and shrinking when food availability is low during winter (Rideout et al., 2006). The highest values for their condition are obtained in late autumn and winter (i.e. prior to the peak spawning), decrease during the spawning season and reach their minimum just after (Alonso-Fernández et al., 2008; Mello and Rose, 2005a). During maturation periods (i.e. late autumn, winter), there is greater demand for energy and therefore Atlantic cod have been observed to have smaller livers with high liver water content (Dutil et al., 2003; Karlsen et al., 2006).

In order to bear part of the energy cost required during the food shortage in winter, Atlantic cod has been shown to feed albeit on a lesser scale in mid-winter (Dutil et al., 2003; Michalsen et al., 2008). In addition, Michalsen et al. (2008) and Schwalme & Chouinard (1999) argue that scarcity of suitable prey could explain the decline in diet consumption during winter season. Several studies have shown that in winter the majority of Atlantic cod have empty stomachs and significantly reduced stomach fullness were also observed (Schwalme and Chouinard, 1999). However, this seems not to be the case for Atlantic cod caught during winter at the windfarm AR (unpublished data). The review of Link et al. (2009) indicated that cod in the North Sea does not alter its preferences for particular prey, and that the consumption of a given prey species by an individual cod is generally proportional to the abundance of the prey. In autumn, storage of mainly lipids in the livers of both fish species was observed while the composition of the muscle remained stable in both seasons.

Lambert & Dutil (1997a) found a correlation between liver lipid content and muscle water content depending on the general physiological condition of the Atlantic cod i.e. whether considered emaciated or normal. Any seasonal change of liver conditions can thus contribute to understand the general conditions such as the wellbeing, historical and present nutritional status and growth of cod (Lambert and Dutil, 1997a).

In the present study we analysed specimens within the size range of 39-46 cm and 20-22 cm for Atlantic cod and pouting, respectively. This indicates that the former are 1.5 to 2 years old (age group I) (Reubens et al., In press-c), while the latter are around 1 year old (Merayo and Villegas, 1994). There are Atlantic cod that mature before the age of 2. However, it may take up to the age of five before all cod mature. Pouting are known to reach their first-maturity around the age of two (Merayo, 1996a). In the present study, the analysed specimens can therefore be classified as juvenile and first-time maturing fishes. For both, wintertime is a period of scarcity in energy accumulation, related to reproductive investment and/or food shortage, in which they have to rely on energy reserves built up during summer and autumn.

In addition to seasonal differences in energy allocation and since E_a/E_c values were >1 in autumn and in spring, we can state that the food intake was higher than the energy required to maintain the fish metabolism and that there was enough energy left for growth and reproduction. This has important consequences for the potential of wind farm artificial reefs as feeding grounds for both fish species. Although we have no detailed information on the prey densities in the sampling site, our data show that both fish species found sufficient energy and thus prey in the sampled wind farm AR. In addition, acoustic telemetry revealed high residency and site fidelity of Atlantic cod near the wind farm artificial reefs (Reubens et al., In press-b), which proves they do not feed occasionally on these reefs.

The proximate composition of the potential prey showed to be similar in the two seasons included in the present study (table 1). However, it is worth mentioning that the amphipod *Jassa* showed a significantly higher lipid content in autumn in comparison to spring. In correspondence to the lipid demands of the fish (see before), it can therefore be considered as a potentially important prey item for both fish species searching for lipid-rich food in autumn. Unfortunately we had no data on the lipid content of *Pisidia* in winter in order to make the same comparison. The overall proximate composition of *Jassa* mats showed to be very low and thus points at a very low nutritional value. Although they form a considerable part of the diet of pouting (Reubens et al., 2011) they are probably consumed while fish reach for *Jassa*.

In terms of FA composition, both *Jassa* and *Pisidia* were found to have a considerable amount of EPA, i.e. $28209 \pm 7304 \mu\text{g/gDW}$ and $17723 \pm 1300 \mu\text{g/gDW}$, respectively. EPA is a PUFA that is listed as essential FA, i.e. higher organisms cannot synthesize it themselves but should gain it from their food sources and at the end from primary producers (Dalsgaard et

al., 2003). Another essential FA and PUFA, DHA, was detected in higher concentrations in *Jassa* ($22734 \pm 995 \mu\text{g/gDW}$) in comparison to *Pisidia* ($6044 \pm 596 \mu\text{g/gDW}$). As potential food source for cod and pouting, they both have the ability to deliver these essential FA to fish.

On the other hand, the FA 14:1 ω 5 was found in high concentrations in *Pisidia* while it was hardly traced in fish muscle and low concentrations were found in fish livers. This can imply that (1) *Pisidia* was not consumed by the fish (but see other FA), (2) that *Pisidia* is preyed upon but that this FA is not assimilated by the fish or (3) this FA was modified by the fish, which could make it a non-biomarker to document the feeding ecology of the fish species under consideration. The first assumption is doubtful as stomach analyses indicated that *Pisidia* and *Jassa* are important food sources for pouting (Reubens et al., 2011) and for cod (Reubens et al., 2013). The typical diatom biomarker 16:1 ω 7 (Budge et al., 2006; Dalsgaard et al., 2003) on the other hand was detected in much higher concentrations in *Pisidia* than in *Jassa* and the mats. This points at a different feeding ecology of both prey species. However, this is of limited relevance for the next trophic level (fish). In this context, the level of essential FA (mainly PUFA) is considered to be more important to unravel the feeding preferences of the tested fish.

For all FA, we found a considerable difference between muscle and liver tissue with the highest FA concentrations in liver. Røjbek et al. (2012) found that mainly the FA composition of the liver reflects the prey of cod. Therefore, the conclusions based on FA profiles of mainly liver can be considered as relevant for the overall feeding ecology of the Atlantic cod and pouting under study.

As in other ecosystems, cod in the North Sea eat available prey within the size range of what they are capable of ingesting (Daan, 1989; Hislop, 1997). Small cod eat mainly crustaceans, gradually increasing the proportion of fish in the diet with ontogeny (Link et al., 2009). Based on stomach analyses, the most important crustacean prey are Caridea (shrimp), Astacidea (lobster), Anomura and Brachyura (crabs) (Daan, 1989; Hislop, 1997). CEA analysis and FA profiles in the present study and stomach content data from Reubens et al. (2011; In press-c) revealed the importance of other benthic species (i.e. *Jassa* and *Pisidia*) in the diet of cod and pouting at the wind farm AR. The AR offers a substrate for these prey species which on their turn provide the necessary energy for higher trophic levels (including fisheries). However, since data on the biomasses of these prey were not available, the turnover rate from prey to fish could not be calculated. Hence, the population size of demersal fishes that could be sustained by the epifauna present at the wind farm AR was not estimated yet. In the present study we analysed Atlantic cod and pouting in the size range of 39-46 cm and 20-22 cm, respectively. The fact that (1) they occur on the wind farm AR, (2) potential prey are abundant there (Kerckhof et al., 2010a) and (3) potential prey have a favourable

proximate composition (this study) generates the conclusion that wind farm AR form a suitable feeding ground for both species. The habitat modification generated by AR should therefore be evaluated as positive, at least in terms of the feeding ecology and probably also the standing stock of both species. To what extent both species potentially compete for the same food source should be further explored. Based on the sufficient energy levels recorded, we have no argument to expect any competition in the wind park AR so far. In contrast to time-consuming stomach analyses that yields merely a snap-shot of the food uptake of fish, the biochemical profiling in the present study provided a more holistic approach to the feeding ecology of the target fishes in terms of energy flow.

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Chapter 8

Productivity at offshore wind farms

Adapted from:

Reubens J, Vandendriessche S, Zenner A, Degraer S, Vincx M, In press. Offshore wind farms as productive sites or ecological traps for gadoid fishes? – Impact on growth, condition index and diet composition. Marine Environmental Research.

ABSTRACT

With the construction of wind farms all across the North Sea, numerous artificial reefs are created. These windmill artificial reefs (WARs) harbour high abundances of fish species. However, in suddenly altered ecosystems, attraction may result in negative ecological consequences for fish. As a result they are caught in an ecological trap. In this paper we investigated whether the wind farms in the Belgian part of the North Sea act as ecological traps for pouting and Atlantic cod. Length-at-age, condition and diet composition of fish present at the windmill artificial reefs was compared to local and regional sandy areas.

Fish data from the period 2009 – 2012 were evaluated. Mainly I- and II-group Atlantic cod were present around the WARs; while the 0- and I-group dominated for pouting. For Atlantic cod, no differences in length were observed between sites, indicating that fitness was comparable at the WARs and in sandy areas. No significant differences in condition index were observed for pouting. At the WARs, they were slightly larger and stomach fullness was enhanced compared to the surrounding sandy areas. Also diet differed considerably among the sites. The outcome of the proxies indicate that fitness of pouting at the WARs was slightly enhanced compared to the surrounding sandy areas. No evidence was obtained supporting the hypothesis that the WARs act as an ecological trap for Atlantic cod and pouting.

Keywords: ecological trap; productivity; fitness; Atlantic cod; pouting; wind power; artificial habitats

1. INTRODUCTION

Offshore wind energy production is an important contributor to the renewable energy production in Europe. All across the North Sea offshore wind farms (OWFs) are planned, being constructed or already operational (Arapogianni et al., 2013; Brabant et al., 2012). With the construction of wind turbines, thousands of artificial reefs (so-called Windmill Artificial Reefs, WARs) will be present in an ecosystem which is naturally composed of soft-bottom sediments. As a result, the OWFs induce some changes in the marine environment which may influence local biodiversity and ecosystem functioning (Andersson et al., 2009).

These artificial reefs often harbour high densities of several benthic and benthopelagic fish species (Leonhard et al., 2011; Reubens et al., 2013; Winter et al., 2010). Two models have been proposed to explain the increased fish abundances (Brickhill et al., 2005). The attraction hypothesis suggests that fish move from the surrounding environment towards the reef. They aggregate at the reef, but there is no net increase in local population. The fish are only concentrated into a smaller area. The production hypothesis on the other hand, assumes that the carrying capacity of the environment increases as a result of the new habitat. More fish are able to settle, survive, grow and contribute to the local population, resulting in net production (both in biomass and in abundance) (Brickhill et al., 2005; Lindberg, 1997; Pickering and Whitmarsh, 1997).

Whether density and biomass increases are the result of attraction or if production is also involved still needs to be resolved in many cases and the outcome depends on a multitude of factors. Different fishes react differently to these new substrates as their dependence upon (artificial) hard substrates varies across species, environment, location and age-specific requirements (Brickhill et al., 2005). Also the reef design, location and abundance of reef units influence fish behaviour (Pickering and Whitmarsh, 1997). In many cases attraction and production are not mutually exclusive and may interact with one another. An initial attraction of a fish species may, in time, turn into production.

To resolve the attraction-production debate, one should focus on the possible consequences of attraction first. Negative ecological consequences for fish may arise from attraction. In suddenly altered ecosystems (as is the case for the construction of offshore wind farms) ecological traps may arise. When an organism is attracted to, and preferably settles in a habitat with suboptimal conditions relative to other available habitats, it is caught in a so-called ecological trap (Robertson and Hutto, 2006). Habitat choices are a consequence of natural selection and are based upon a number of ecological cues which indicate the quality status of a habitat (Schlaepfer et al., 2002). An ecological trap may occur when changes in the environment act to uncouple the cues used to assess habitat quality from the true quality of the environment (Robertson and Hutto, 2006). In the marine environment, fish aggregation devices for instance are known

to have the potential as ecological traps (Hallier and Gaertner, 2008). Although, they also may have the potential to increase the condition and reproductive outcome of fishes (Dempster et al., 2011).

In the Belgian Part of the North Sea (BPNS) the seabed is dominated by soft-bottom sediments, while natural hard substrates are rare (Mallefet et al., 2008). In recent years, the construction of OWFs created numerous WARs.

Atlantic cod (*Gadus morhua*) and pouting (*Trisopterus luscus*) are two gadoid species that often occur at these WARs in the BPNS (Reubens et al., 2013). Atlantic cod is a benthopelagic fish species that occurs in the North Atlantic Ocean. It is widely distributed throughout the North Sea in a variety of habitats and is a highly valued commercial species, severely suffering from overexploitation (ICES, 2012a). Pouting is also a benthopelagic fish, but smaller than cod and occurring from the Skagerrak to the African coast. It lives in soft sand or rocky areas and often occurs in large schools (Merayo and Villegas, 1994). It is a commercial species in southern European countries (Merayo, 1996b).

Both species are known to be attracted to the WARs in the BPNS and high catch rates are observed during summer and autumn (Reubens et al., 2013; Reubens et al., 2011). In addition, acoustic telemetry revealed that Atlantic cod is highly resident at WARs (Reubens et al., In press-b). However, whether the WARs are poorer or richer in habitat quality than the surrounding soft-bottom sediments remains unknown. Each habitat has its own community structure and carrying capacity, influenced by environmental parameters (e.g. currents, heterogeneity, temperature, sediment type). As a result, habitat selectivity influences the fitness of the associated fishes. Fitness is represented by growth, condition index and diet; and all three may differ or be affected by the environmental differences among habitats.

In this paper we investigated growth, condition and diet (i.e. proxies for fitness) of pouting and Atlantic cod at different sites in the North Sea. Proxy values at the WARs were compared to values from sandy areas to resolve whether the WARs act as ecological traps for these species. The values were compared locally (i.e. close to the WARs), and regionally (i.e. BPNS and ICES area IVc).

2. MATERIAL & METHODS

2.1 Study sites and data collection

Pouting and Atlantic cod were sampled from January 2009 until December 2012 at WARs and at sandy areas (i.e. Goote Bank, BPNS and ICES area IVc). The WARs under investigation are located at an OWF in the BPNS (Fig. 1), more precisely at the Thorntonbank, a natural sandbank 27 km offshore (coordinates WGS 84: 51°33'N –

2°56'E) (C-Power concession). Water depth varies between 18 and 24 m in the wind farm. It consists of 54 wind turbines (spread over 18 km²), with two types of foundations: concrete gravity based (6 turbines) and steel jacket foundations with four legs (48 turbines). Both function as WARs.

All fish from the WARs were caught at the gravity based foundations. These foundations have a diameter of 15 metres at the seabed, at a depth of about 22.5 m at mean low water spring (MLWS). They are surrounded by a scour protection layer of pebbles and rocks with a maximum width of 18 m. The total surface area of the hard substrates (turbine foundation and scour protection together) is approximately 2043 m² (Peire et al., 2009). The surrounding soft sediment is composed of medium sand (mean median grain size 374 µm, SE 27 µm)(Reubens et al., 2009).

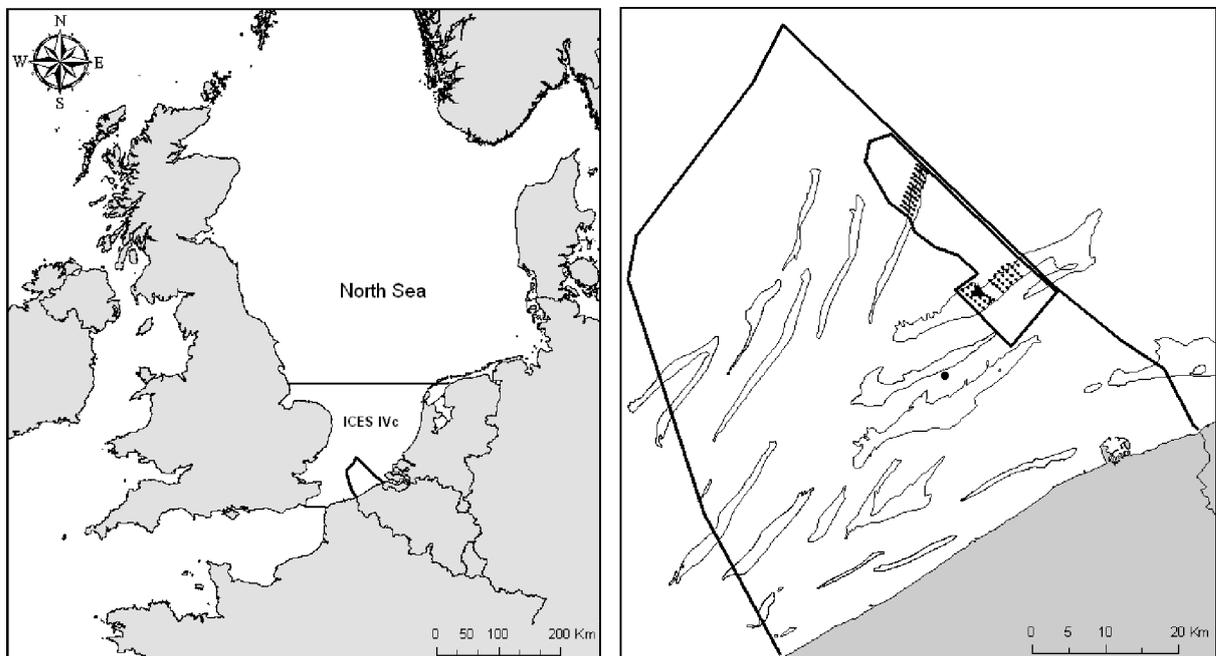


Figure 1. Left panel: Map of the North Sea with indication of ICES area IVc and the Belgian part of the North Sea (BPNS); Right panel: detailed view of the BPNS with delineation of the windmill zone and indication of the installed wind turbines. Sampling locations at the Windmill artificial reefs (black triangle) and the sandy area (black circle) are indicated.

The sandy area sampled for local comparison (called Goote Bank hereafter) is located at the Goote Bank (coordinates WGS 84: 51°27'N – 2°52'E) at approximately 25 m depth at MLWS. The area is composed of medium sand (mean median grain size 371 µm, SE 83 µm) (Reubens et al., 2009). For regional comparisons, data concerning Atlantic cod and pouting were extracted from the databases from the institute for Agricultural and Fisheries Research (ILVO). Data from the BPNS (Aquatic Environment Database) and the ICES area IVc (Fisheries Biology Database) were used. Both the BPNS and the Southern bight (i.e. ICES area IVc) are characterised by a complex system of sand banks, which are orientated parallel to the coasts. The seabed consists mainly of medium to fine-grained

sands, but mud deposits are present as well. The BPNS is a small part of the North Sea (only 3600 km²), it is a shallow sea with an average depth of 20 m and maximum depth of 46 m. The Southern Bight of the North Sea is bordered by France, Belgium, The Netherlands and the UK (Fig.1). It is delimited to the north around 53°N and to the south by the Dover Strait. The Southern bight has a maximum depth of 54 m. (Degraer et al., 2006; Jones et al., 2004; Kerckhof and Houziaux, 2003; Merckx, 2011).

At the WARs and the Goote Bank fishes were collected by standardized line fishing (hooks: Arca size 4; bait: *Arenicola marina*). Sampling was restricted to daytime hours. Fishing time was 45 min. on average and the number of fishermen ranged from 2 to 8. At the WARs, angling was performed 1 to 10 metres away from a turbine (i.e. within the erosion protection layer radius) just above the bottom of the seabed, assuring catching WAR associated individuals. The fish were measured (total length) and weighed (wet weight), and their stomachs were removed and preserved in an 8% formaldehyde-seawater solution.

At the BPNS, fish were caught with an 8-metre beam trawl with a fine-meshed shrimp net (stretched mesh width 22 mm in the cod end) and a bolder-chain. The net was dragged for 30 min. on average at an average speed of four knots over the bottom. For ICES area IVc data from both research and commercial vessels were used. Research vessels (bottom trawl surveys) used a 4-metre beam trawl with a stretched mesh width of 40 mm in the cod end. The net was dragged for 30 minutes on average at an average speed of four knots over the bottom. Commercial vessels used nets with a stretched mesh width between 80 and 220 mm and tows varied between 40 min. and six hours at an average speed of four to seven knots. All fish were measured for total length.

Fulton's condition index was used as an indicator of the general condition of the fish. It was calculated as $F = (W/TL^3) * 100$, where W = total weight (g) and TL = total length (cm). The use of somatic weight (total weight minus gonad and stomach content weights) instead of total weight would have provided a more accurate reflection of condition, since feeding intensity and gonad maturation may vary significantly (both temporally and regionally) (Lambert and Dutil, 1997b). Unfortunately data on somatic weight was mostly not available.

To quantify the feeding rate and investigate the prey composition in the diet, stomach content analyses were performed for pouting. All food components in the stomachs were identified to the lowest possible taxonomic level. Wet weight, dry weight (60 °C for 48 h) and ash-free dry weight (500 °C for 2 h) were measured for all separate food contents in each stomach. Prey were later categorized in 10 groups: Anthozoa, Mysida, Amphipoda, Natantia, Reptantia, Mollusca, Echinodermata, Pisces, detritus and rest.

To investigate the patterns in feeding rates a stomach content fullness index (I_F) was calculated for each stomach of pouting as $I_F = 10\,000cL^{-3}$, where c is the stomach content mass (wet weight, g) and L is the fish length (total length, cm). Dietary composition was assessed by the frequency of occurrence (%FO) and gravimetric abundance index (%G). For the gravimetric analysis ash-free dry weight was used.

$$\%FO_i = (N_i/N) * 100$$

$$\%G_i = (\sum S_i / \sum S_a) * 100$$

N_i is the number of predators with prey type i in their stomach, N the total number of non-empty stomachs, S_i is the stomach content composed by prey i and S_a the total stomach content of all stomachs together (Amundsen et al., 1996). Only non-empty stomachs were used in the analysis as regurgitation could have occurred while fish were being hauled.

Table 1. Overview of data available (X) per region. Data are from the period 2009-2012. For pouting no data was available from ICES area IVc; for Atlantic cod there was not sufficient data from the sandy area available for proper analysis.

	Pouting				Atlantic cod			
	WAR	Sandy area	BPNS	IVc	WAR	Sandy area	BPNS	IVc
Length	X	X	X		X		X	X
Condition	X	X			X			
Diet	X	X			X			

As shown by table 1, not all analyses could be performed for each region. For the data from the BPNS and ICES area IVc, only information on length of the fish was available; as a consequence nor condition, neither fullness index could be calculated for these fishes. For Atlantic cod, data for the local comparison (Goote Bank) were too sparse; due to the low catch rates and a scattered distribution throughout the year.

Only for the local comparison for pouting all three analyses (i.e. length-at-age, condition and diet composition) could be performed. For Atlantic cod and for the other regions only length-at age information was available (Table 1). However, it was decided to use this data as well as each analysis provided useful information and contributed to resolve the main question of the study.

2.2 Data analysis

Length-frequency distributions were built to investigate the age composition in the population. It gives clear information concerning the age groups present and their length distributions. The younger age groups usually do not have much overlap in length distribution (Daan, 1974), which makes it easier to distinguish the different age groups.

No otolith information was available for data from the WARs, Goote Bank and BPNS. However, based on the length-frequency distributions the different cohorts of Atlantic

cod and pouting present could be clearly separated. Length-at-age keys helped to correctly assign the cohorts to their age group. The key for Atlantic cod was compiled from data from the ICES area IVc for the period 2009-2012 (Table 2). For pouting, length-at-age information (Table 3) was based on Merayo and Villegas (1994) and the regression from standard length to total length ($TL = 2.35 + 1.102 SL$) was based on Hamerlynck and Hostens (1993).

Separate length-frequency distributions were made for Atlantic cod and pouting from the WARs, Goote Bank and BPNS. For Atlantic cod from ICES area IVc otolith information was available for age determination.

The comparison of length and condition index between sites was separated per age group.

Table 2. Average length-at-age per quarter for Atlantic cod, based on data from the ICES area IVc in the period 2009-2012. Age was determined by otolith analysis. Values are expressed as total length (cm). Q1 = Jan-Mar, Q2 = Apr-Jun, Q3 = Jul-Sep, Q4 = Oct-Dec. The numbers between brackets indicate the number of fish available for age determination.

	Q1	Q2	Q3	Q4
0 year			16.8 (6)	19.9 (71)
1 year	26.5 (39)	29.7 (173)	35.2 (127)	43.3 (715)
2 year	49.3 (184)	51.0 (925)	53.2 (262)	54.6 (594)
3 year	64.1 (180)	68.3 (283)	65.0 (13)	61.2 (687)

Table 3. Average length-at-age for pouting (for both sexes). Data are based on Merayo and Villegas (1994) and expressed as total length (cm). Values refer to length at the end of the year. Regression from standard length (SL) to total length (TL) is based on Hamerlynck and Hostens (1993); $TL = 2.35 + 1.102 SL$.

	Male	Female
0 year	20.7	21.4
1 year	27.5	26.8
2 year	31.5	33.1
3 year	35.6	38.2

To investigate differences in fish size and Fulton's condition index between sites and months/seasons PERMANOVA was used. PERMANOVA makes no explicit assumptions regarding the distribution of original variables. Univariate and multivariate ANOVAs were performed with p-values obtained by permutation (Anderson et al., 2008). To investigate the spatio-temporal effects (i.e. sites and period) a 2-factor design was used with fixed factors location (loc) and period. Depending on the data, the period used was month (mo) or season (se). As the design was unbalanced at the lowest level (i.e. unequal numbers of replicate samples within each factor level of the design), it was decided to use Type I sums of squares in the analyses (Anderson et al., 2008). The lack of balance means that

the various interactions and main effects cannot be estimated independently, and thus the outcome will depend on the order in which the factors are entered in the model (Anderson et al., 2008). For this study, the order used is location - month. An Euclidian distance similarity matrix was used (Clarke et al., 2006). In case of significant factor effects, pair-wise tests were performed to investigate which groups within a factor were significantly different. In case of significant interactions, pair-wise tests within the interaction were performed. Homogeneity of dispersions was tested with PERMDISP, using distances between centroids. Data were square root transformed if dispersion effect was significant. If PERMDISP was significant, even after transformation, results were interpreted with care.

To investigate the feeding strategy of pouting and to compare dietary compositions among sites, non-parametric multivariate techniques were used. All multivariate analyses were performed on prey biomass (ash-free dry weights). Prior to analysis, fourth root transformations were done to weigh the contributions of common and rare dietary categories in the similarity coefficient (Dempster et al., 2011). The similarity matrix was constructed using the Bray-Curtis index of similarity. Non-metric multidimensional scaling (MDS) was used as ordination method. The analysis of similarity (ANOSIM) was performed to investigate differences among sampling locations, while similarities within groups and dissimilarities between groups were calculated using similarity percentages (SIMPER).

The non-parametric Mann-Whitney U test was used to compare stomach content fullness between locations. This statistical analysis was performed in R 2.15.1 software (www.r-project.org).

Multivariate analyses and PERMANOVA were performed in the Plymouth routines in multivariate ecological research (PRIMER) package, version 6.1.11 with PERMANOVA add-on software (Anderson et al., 2008; Clarke and Gorley, 2006). A significance level of $p < 0.05$ was used in all tests. Results are expressed as mean \pm standard deviation (SD).

3. RESULTS

3.1 Community structure: length and age distribution

Over the four years' sampling period, 549 and 40 Atlantic cod were caught at the WARs and Goote Bank respectively (Table 4). Lengths were ranging from 19.8 - 61.6 cm and weights from 70 - 2320 g at the WARs, while at the Goote Bank lengths were ranging from 20.7 - 40.6 cm and weights from 90 - 650 g. For pouting, 824 and 96 specimens were caught at the WARs and Goote Bank respectively with lengths ranging from 12.5 - 38.5 cm and weights from 35 - 620 g at the WARs. Length ranged from 13 - 22.4 cm and weights from 30 - 150 g at the Goote Bank.

The length-frequency distribution at the WARs clearly revealed that the I-group of Atlantic cod was present year round (Fig. 2). Some II-group individuals were present as well, although in much lower numbers and mainly during the first half of the year. Thereafter, they were only sporadically encountered. At the Goote Bank only few Atlantic cod were caught and their distribution was scattered over the year. Most of them belonged to the I-group. As only few fish were caught, clear length distributions could not be obtained.

In the BPNS, 215 Atlantic cod ranging from 10 up to 93 cm were caught in this period (Table 4). I-group cod dominated the catches in spring, but the II- and III-groups were represented as well. Some older specimens were observed, although in very low numbers. In autumn, the 0-group dominated the catches.

In the ICES fishing area IVc, Atlantic cod up to 5 years old were observed. Age group I, II and III dominated the catches. In autumn, the 0-group was observed as well. In this area 4384 specimens were caught and length ranged from 15 up to 98 cm (Table 4).

Table 4. Overview of the number of specimens available in the dataset per region for each quarter (all age groups). Q1 = Jan-Mar, Q2 = Apr-Jun, Q3 = Jul-Sep, Q4 = Oct-Dec. *Data collected in Sep and Oct.

	Pouting				Atlantic cod			
	WAR	Sandy area	BPNS	IVc	WAR	Sandy area	BPNS	IVc
Q1	55	6	30	-	38	16	104	418
Q2	164	0	-	-	225	18	-	1406
Q3	238	19	-	-	188	6	-	409
Q4	367	71	478*	-	98	3	111*	2151
Total	824	96	508	-	549	43	215	4384

For pouting, both the 0- and I-group were observed at the WARs (Fig. 2). The first sightings of the 0-group were in August/September. The I-group was present year round, though was only well represented from May until October. At the Goote Bank pouting was mainly observed from September until December and only the 0-group was encountered.

In the BPNS, 508 pouting specimens were caught, ranging from 6 to 23 cm. The individuals caught in autumn all belonged to the 0-group, while in spring only the I-group was encountered. No information was available for the ICES IVc region.

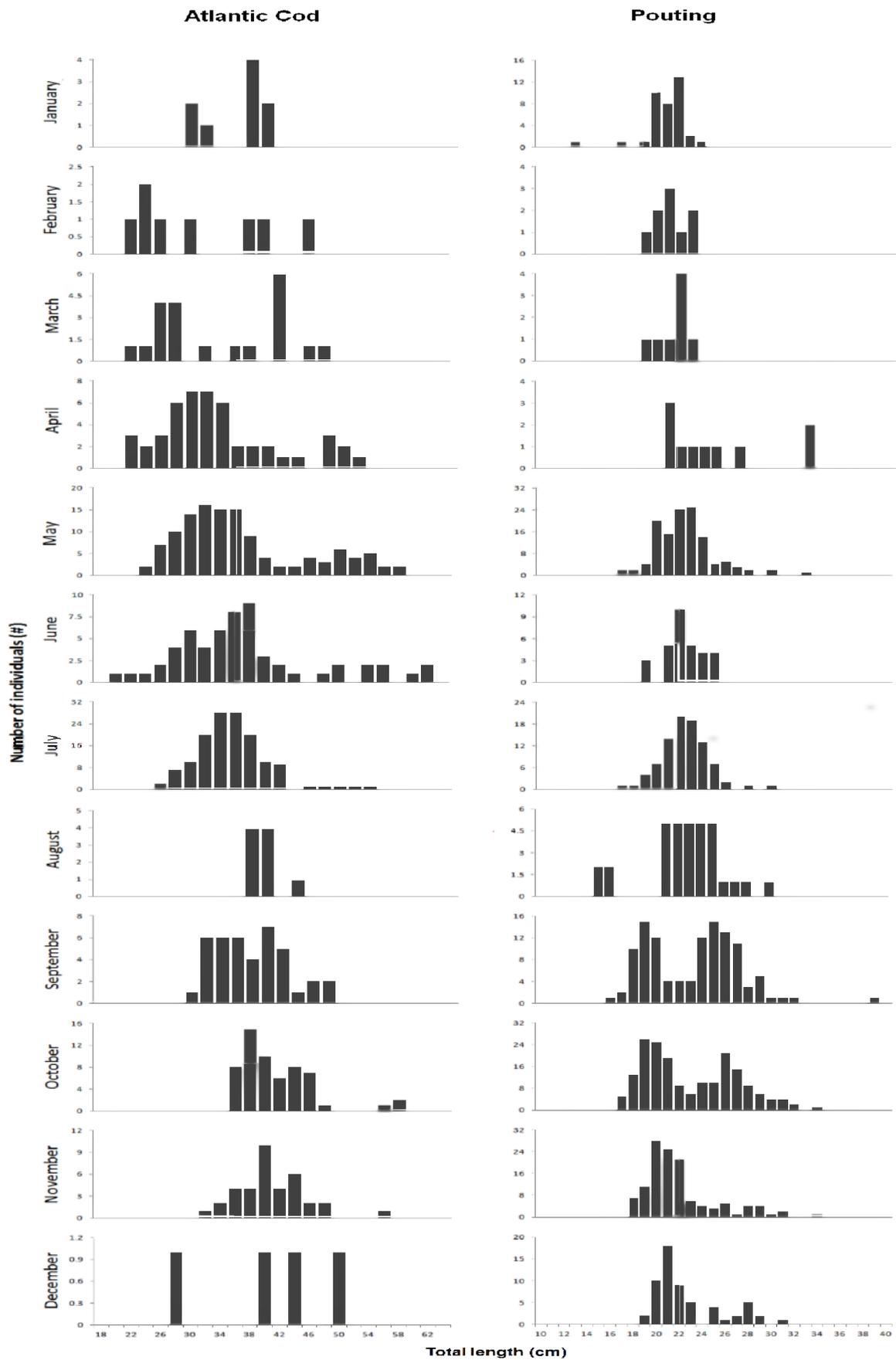


Figure 2. Length-frequency distribution of Atlantic cod and pouting at the WARs. Values are expressed as total length (cm). Mind the differences in scale.

3.2 Size comparison

Our data did not allow to compose growth curves. Only one age group was well represented and not enough data points were available from each sampling. As a result no good fit was obtained in the growth models. However, the length-frequency distributions showed some trends in growth at the WARs. For Atlantic cod, growth was observed throughout the year and was somewhat faster during winter months (Fig 2). For pouting on the contrary, almost no growth was observed during the winter months. From May onwards, average length increased slightly per month. For both the 0-group and I-group an increase in average length was observed over time.

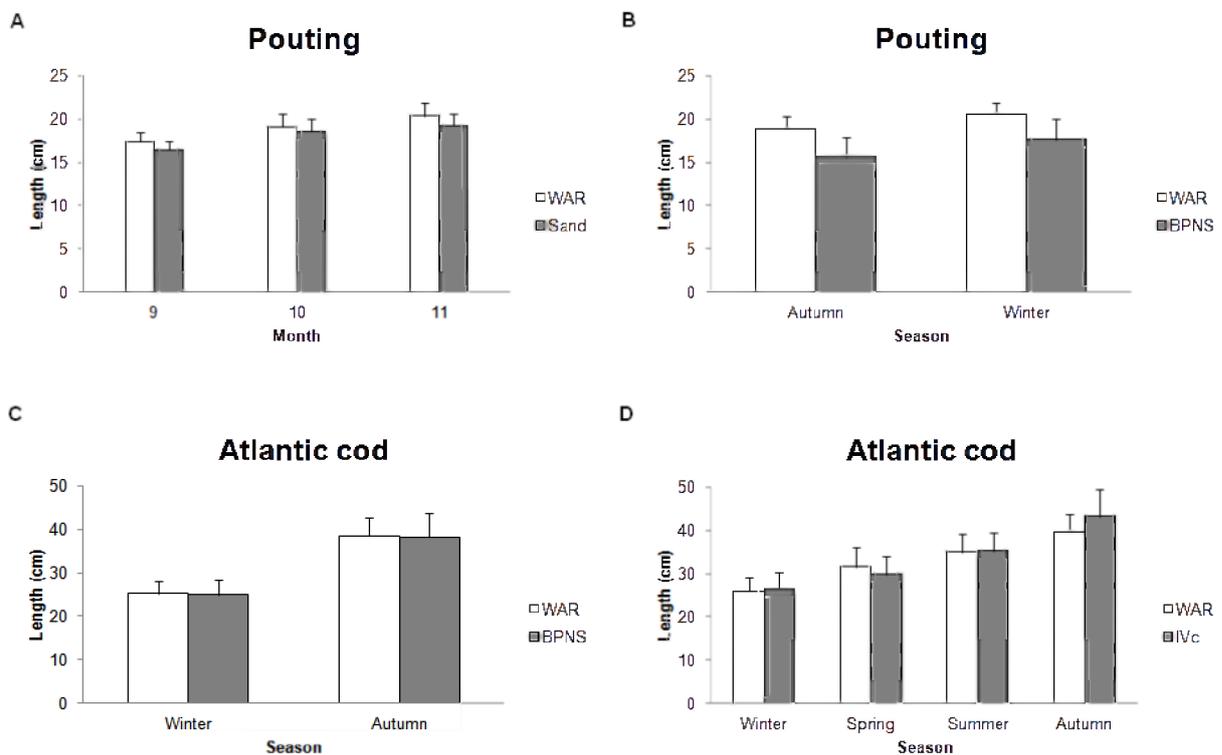


Figure 3. Comparison of average total length (cm) + SD of pouting and Atlantic cod between the different regions. (A) pouting at WARs and sandy area, (B) pouting at WARs and BPNS, (C) Atlantic cod at WARs and BPNS, (D) Atlantic cod at WARs and ICES area IVc.

For Atlantic cod, no comparison in length between the WARs and the Goote Bank could be made due to the low catches at the sandy area (i.e. local comparison). However, a comparison was made between the WARs and the soft substrate sediments of the BPNS for the I-group. There were 89 and 111 individuals caught at the BPNS and the WARs respectively. At the sand, 45 cod were caught in spring and 44 in autumn. At the WARs, this was 16 and 95 respectively. The Atlantic cod at the sandy substrates (24.9 ± 3.3 cm in spring and 38.2 ± 5.5 cm in autumn) had a comparable size to the ones at the WARs (25.1 ± 2.8 cm and 38.5 ± 4.1)(Fig. 3). However, both between sites ($p = 0.0001$) and between seasons ($p = 0.0001$) length was significantly different. No interaction effect was observed

($p = 0.9$). Permdisp was significant for location (Table 5), which may explain the significant differences in length, notwithstanding the comparable sizes.

Likewise, length comparison was done between the WARs and the ICES area IVc (seasonally) for the I-group (Fig.3). From these sites, 473 and 1054 individuals were analysed respectively. Significant loc x se ($p=0.0001$) interactions in the model demonstrated that differences in length between habitats was influenced by season. Pair-wise comparisons revealed that only in quarter 2 ($p = 0.0002$) and 4 ($p = 0.0001$) significant differences in length were observed between the sites. However, it should be noted that PERMDISP was significant as well (Table 5). In quarter 2 average length was higher at the WARs, while in quarter 4 it was highest in area IVc.

A comparison in length of pouting was made between the WARs and the Goote Bank. Only the 0-group during September-November was analysed in order to have sufficient data in both sites. Significant differences in length were revealed, both between sites ($p = 0.0001$) and between months ($p = 0.0001$). No interaction effect was observed (Table 5). At the WARs fish were bigger compared to the Goote Bank in all months (Fig 3). For both sites length increased over months. For the I-group, no comparison was possible as this age group was not encountered at the sandy area.

A comparison in length of pouting was made between the WARs and the soft substrate sediments of the BPNS (Fig. 3). In total 508 pouting were caught in the BPNS. Only 30 individuals were caught in spring, while 478 were caught in autumn. For the WARs 158 individuals could be used for comparison; 8 and 150 individuals for spring and autumn respectively. For the comparison in autumn fish belonged to the 0-group, while in spring they already belonged to the I-group. Significant differences in length were revealed, both between sites ($p = 0.0001$) and between seasons ($p = 0.0001$). No interaction effect was observed ($p = 0.24$). PERMDISP was significant for location (Table 5). At the WARs pouting were bigger (18.8 ± 1.5 and 20.5 ± 1.4 cm) compared to the BPNS (15.6 ± 2.3 and 17.6 ± 2.4 cm) in autumn and spring respectively.

No comparison could be made for ICES area IVc.

3.3 Condition and Diet analysis – pouting

For pouting, the condition index could be compared between the WARs and the Goote Bank in late summer and autumn, but no significant differences were detected ($p = 0.21$) between the sites. However, condition differed significantly between September - October and September - November ($p = 0.06$ and $p = 0.07$ respectively). In September the Fulton condition index was somewhat lower compared to the other months (Fig. 4). Condition could not be investigated for the regional sites because no information on individual weight was available.

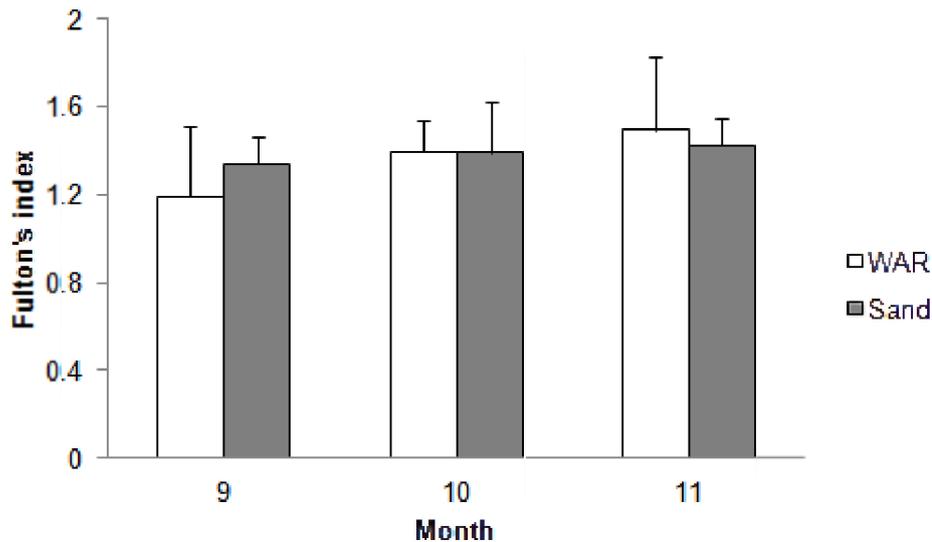


Figure 4. Average fulton's condition index (+SD) of pouting at the WARs and the sandy area.

Stomach contents were compared between the WARs and the Goote Bank for pouting caught in October-November 2011. There were 43 and 17 stomachs analysed for the WARs and the Goote Bank respectively. One empty stomach (2.3 %) was present at the WARs, none at the sandy area. However, the average stomach fullness was significantly higher (non-parametric Mann-Whitney U test, $p = 0.02$) at the WARs (1.5 ± 1.4) compared to the Goote Bank (0.6 ± 0.8). The 2-dimensional MDS plot and Simper analysis, based on ash-free dry weights of the prey groups, revealed that the diets of fish from the WARs were more clustered and similar (average similarity 59 %), while the ones from the Goote Bank had a more diverse composition (average similarity 37 %). ANOSIM indicated that significant differences in diets were present between the two sites ($R = 0.5$, $p = 0.0001$). The diets of pouting at the WARs were dominated by Amphipoda, followed by Reptantia, while the fish at the Goote Bank were characterized by more diverse diets with Pisces, Reptantia, Anthozoa and Amphipoda as the most important prey groups (Table 6). A more detailed analysis of the individual prey species showed that pouting at the WARs mainly fed upon hard substrate associated prey species (i.e. *Jassa herdmani*, *Pisidia longicornis* and *Liocarcinus holsatus*), while at the sandy area they fed both on hard and soft substrate associated prey species (i.e. *Callionymus* sp., *Actiniaria* sp., polychaeta sp. and *L. holsatus*) (Table 6).

Table 5. PERMANOVA results from the main test for Atlantic cod and pouting. P-Values obtained by permutation. Factor 'Period' denotes months or seasons, depending upon the data. * PERMDISP significant; ** Square root transformed.

		Location				Period				Location x Period			
		df	MS	Pseudo-F	p	df	MS	Pseudo-F	p	df	MS	Pseudo-F	p
Pouting	length (local)	1	88.5	43.7	0.0001	2	90.5	44.7	0.0001	2	1.1	0.5	0.6
	length (BPNS)**	1	18.6	240	0.0001*	1	2	25.7	0.0001	1	0.02	0.26	0.6*
	Fulton index **	1	0.01	1.6	0.2	2	0.05	5.2	0.007	2	0.02	1.8	0.2
Cod	length (BPNS)**	1	1478.7	93.9	0.0001*	3	2214	140.6	0.0001*	3	20	1.3	0.3
	length (ICES IVc)**	1	57	315.2	0.0001*	3	93.6	517.5	0.0001*	3	2.6	14.4	0.0001*

Table 6. Overview data from stomach content analysis from the Goote bank (sand) and the WARs. The gravimetric abundance index (% G) and frequency of occurrence (%FO) of prey groups present in the stomachs of pouting are listed in the left column. In the right column the 5 most important prey species (in terms of weight) are listed.

	% G		%FO		% G			
	Sand	WAR	Sand	WAR	Sand		WAR	
Amphipoda	8.1	66.8	64.7	94.1	<i>Callionymus</i> sp.	43.14	<i>Jassa herdmani</i>	61.97
Anthozoa	9.7	0.0	5.9	0.0	Pisces spec.	9.82	<i>Pisidia longicornis</i>	10.22
Detritus	0.1	0.3	5.9	5.9	Actinaria sp.	9.69	Pisces sp.	8.42
Echinodermata	0.1	0.0	5.9	0.0	Polychaeta sp.	4.72	<i>Liocarcinus holsatus</i>	5.45
Mollusca	0.1	0.0	17.6	5.9	<i>Liocarcinus holsatus</i>	4.28	<i>Necora puber</i>	3.05
Mysidacea	1.4	0.0	11.8	5.9				
Natantia	4.4	0.3	29.4	2.9				
Pisces	53.0	8.4	23.5	5.9				
Reptantia	15.8	22.9	76.5	79.4				
Rest	7.4	1.2	76.5	55.9				

4. DISCUSSION

We demonstrated that at the investigated WARs specific lengths and age groups were present in the catches of Atlantic cod and pouting. For Atlantic cod this was the I-group, while for pouting the 0- and I-group dominated the catches. Although not represented in the samples, other age groups may be present as well at this habitat. The smallest cod observed measured 20 cm, and nearly all pouting were larger than 15 cm. Scuba divers performing visual surveys (Reubens et al., 2011) on the other hand, observed Atlantic cod as small as 5 cm and pouting of 10 cm at the WARs. This suggests that other age groups are present but not efficiently caught using hook and line. Line fishing is a selective fishing technique and type and size of baits, hook design, fishing strategy and fish ecology all may influence the species and size selectivity (Løkkeborg and Bjordal, 1992). Prey preferences of fish are related to prey size (Daan, 1973), thus size of bait and hooks will influence the fish size caught. In this study we used small hooks (Arca nr 4) and live bait (the lugworm *Arenicola marina*). The size of the hook induces a lower and upper limit of fish sizes able to be caught. For small fish the hook may be too big, while big fish may no longer be efficiently hooked (Løkkeborg and Bjordal, 1992).

In beam trawl fishing mesh size, configuration and number of meshes in the circumference and towing speed influence fish sizes caught (Campos et al., 2003; Herrmann et al., 2007); however, it is still much less selective compared to line fishing. This may partly explain the difference in age groups present in the catches. In the trawl catches Atlantic cod from 0 to 5 years old were observed.

It is notable that at the soft substrates (both locally and nationally) only the 0-group of pouting was observed in autumn. At the WARs the I-group was present as well. This suggests that larger individuals need hard substrates as they were not encountered at the sandy areas but dominated at the WARs. Literature information (Merayo, 1996a) confirms this result.

It was shown that pouting at the WARs is somewhat larger compared to the ones at the sandy substrates, while Fulton's condition index was comparable between both habitat types. To achieve growth, the food intake should be higher than the energy required to maintain the fish metabolism. The energy required for metabolism in turn depends on a multitude of environmental factors (e.g. food availability, currents, temperature). If environmental conditions allow optimal energy gain, more energy will be available for growth. Løkkeborg et al. (1999) showed that, although Atlantic cod may feed throughout the 24 h cycle of a day, their feeding behaviour is expected to reflect a style resulting in this optimal energy gain. As environmental factors differ between habitats, the energy gain may differ per habitat. At the WARs many hiding places against currents and predators are present, reducing energetic costs of swimming. In addition, a lot of potential prey species are available in high densities (Reubens et al., In press-a). This may

explain the differences in length-at-age of pouting between the WARs and the sandy bottom sediments.

For Atlantic cod, significant differences in size were revealed between the WARs and both the BPNS and ICES area IVc, despite the comparable average lengths. In the PERMANOVA model the assumption for homogeneity of dispersions was not fulfilled for loc x se; PERMDISP had a p-value below 0.05. This means that a significant result is caused by a significant difference in dispersion or some combination of location and dispersion between the groups (Anderson et al., 2008). As average lengths were comparable (Fig. 3), differences were probably related to the dispersion effect.

Although only few stomachs from individuals caught at the Goote Bank were available for analysis, some interesting results were obtained concerning the diet of pouting. Average stomach fullness was much elevated at the WARs compared to the Goote Bank and diet composition differed significantly between both sites. It is striking that at the WARs all dominant prey items were associated with the hard substrates. These prey species occur in very high densities (up to 20,000; and 4,000 ind/m² for *J. herdmani* and *P. longicornis* respectively) in this habitat (Kerckhof et al., 2010b). Pouting from the Goote Bank on the other hand had a more diverse diet and similarity in dietary items was much lower within this habitat compared to the WARs. They both fed on hard and soft substrate associated prey, which may be an indication that these specimens have to spend more energy for sufficient food intake as they move forth and back between hard and soft substrates. The lower fullness index signifies the lower food availability in this habitat. A recent study (Reubens et al., 2011) on the stomach content analysis of pouting caught near the wind farms in the BPNS revealed that the tube building amphipod *J. herdmani* and the long-clawed porcelain crab *P. longicornis* are the most important prey species, which is confirmed in this study. Though they are dominant in the diet, they are not necessarily a profitable energy source. Therefore, De Troch et al. (Submitted) have calculated the proximate composition (i.e. quantifying the levels of carbohydrates, lipids and proteins in the tissue in order to determine the total available energy) for both prey species. Especially *J. herdmani* had a high proximate composition and thus energy available. It is a lipid-rich food source. Other than energy requirements, fish also have fatty acid requirements. Mainly essential fatty acids are important, as higher organisms cannot synthesize these themselves but should gain them from their food sources (Dalsgaard et al., 2003). Both in *J. herdmani* and in *P. longicornis* a considerable amount of essential fatty acids (i.e. EPA and DHA) were found (De Troch et al., Submitted) rendering them potentially important prey types.

Based on the information of the current study no evidence was obtained to assume that the WARs act as an ecological trap for Atlantic cod and pouting (related to habitat

quality). For Atlantic cod length was comparable to the length of individuals at the local and regional reference sites. As a result, it may be concluded that the fitness of Atlantic cod at the WARs was similar to the one at the sandy reference areas. Length of pouting at the WARs was slightly larger compared to individuals at the sandy areas, while no significant differences in condition were observed between sites. In addition, food was plentiful at the WARs and no restrictions related to sufficient food intake were encountered. Based on the measured proxies, fitness of pouting was even slightly better compared to the sandy areas (increased length and enhanced fullness index). This might be a first indication towards production (i.e. increased biomass) of pouting at the WARs. It should be annotated that the current results do not exclude the WARs to act as an ecological trap via increased fishing mortality. Fish aggregations are particularly vulnerable to fishing pressure (Rose and Kulka, 1999). Concentration of both fish and fisheries activities can lead to local overfishing. If uncontrolled fisheries would be allowed at the WARs, fish aggregating in this habitat would experience enhanced mortalities (i.e. fishing mortality), and they would thus be caught in an ecological trap. However, so far the wind farm concession areas in the BPNS exclude all fisheries activities. As a consequence, fish aggregating in this habitat are released from exploitation pressure; resulting in higher survival chances (i.e. less fishing mortality). This might lead to an increasing production of the population, aside from fitness issues.

In this study, only one wind farm was sampled. In ideal conditions replicates should be taken to investigate life history traits of Atlantic cod and pouting, but sampling multiple wind farms is logistically challenging. However, the selected sandy reference sites are unlikely to vary considerably in environmental factors (i.e. temperature, salinity, current conditions, sediment characteristics) from the wind farm.

The integration of data from local, national (BPNS) and regional (ICES area IVc) levels allowed for appropriate data interpretation on a broader scale, increasing the relevance of the study. However, more information on condition and diet composition (mainly for Atlantic cod) from fish caught at the sandy areas is highly recommended.

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Belgian wind farm monitoring programme, with the financial support of C-Power nv, Belwind nv and Northwind nv.



Part IV.

General discussion: an overview of four years research



Synthesis of four years of research – where are we now?

Adapted from:

Reubens J, Degraer S, Vincx M, Submitted. The ecology of benthopelagic fishes at offshore wind farms – a synthesis of four years of research. Environmental Research Letters.

ABSTRACT

In recent years offshore wind farms arose all across the North Sea and the numerous wind turbine foundations add a significant amount of artificial reefs (so-called windmill artificial reefs, WARs) to the environment. These WARs induce changes in the marine environment, having an influence on local biodiversity and ecosystem functioning. In this study, we focused on the effects on benthopelagic fish. From 2009 until 2012 the behavioural ecology of Atlantic cod (*Gadus morhua*) and pouting (*Trisopterus luscus*) was investigated at WARs in the Belgian part of the North Sea. Information on length-frequency distribution, diet, community structure and movements of both species were combined to gain insights on their behavioural ecology and to unravel whether production occurs at WARs. The acquired knowledge was integrated and discussed in relation to ecological processes and fisheries activities that may take place in offshore wind farms.

We demonstrated that specific age groups of Atlantic cod and pouting are seasonally attracted towards the windmill artificial reefs, that they show high residency and site fidelity and feed upon the dominant epifaunal prey species present. Growth was observed throughout the period the fishes were present. As a result, production on a local scale can be assumed. So far, no changes in production were observed on a regional scale.

Based on the current knowledge on the ecology and population structuring of Atlantic cod and pouting at the WARs, we judged that no fisheries activities should be allowed inside the offshore wind farms in the Belgian part of the North Sea.

Keywords: Atlantic cod, benthopelagic fish, offshore wind farm, pouting, reef effects, windmill artificial reef

1. INTRODUCTION

1.1 Offshore wind farms as windmill artificial reefs

In recent years offshore wind farms (OWFs) arose all across the North Sea and most bordering countries are planning a further large scale development in the North-East Atlantic Ocean (Arapogianni et al., 2013; Wilhelmsson and Malm, 2008).

The numerous wind turbine foundations deployed on the seabed of the North Sea add a significant amount of artificial reefs (so-called windmill artificial reefs, WARs) to the environment. These WARs (and on a larger scale the OWFs) induce changes in the marine environment which have an influence on local biodiversity and ecosystem functioning (Andersson et al., 2009). As a consequence, the OWFs have environmental costs and benefits (Langhamer et al., 2009). One of the possible changes in the ecosystem is the attraction of fishes towards WARs; they often harbour high local densities of several benthopelagic fish species (Andersson et al., 2009; Leonhard et al., 2011; van Deurs et al., 2012; Winter et al., 2010). However, whether the fish are merely attracted or also production occurs is still subject to debate. This issue is known as the 'attraction-production debate' (Lindberg, 1997).

1.2 Attraction-production debate extended with ecological traps

The attraction-production issue was first raised during the Third International Artificial Reef Conference in 1983 (Lindberg, 1997). Artificial reefs (ARs) had become a popular tool to concentrate fishes, resulting in enhanced catches in both commercial and recreational fisheries (Bohnsack and Sutherland, 1985; Polovina, 1989, 1991). It was assumed that most reef fish stocks were limited by their available habitat (Bohnsack, 1989). Increasing the amount of suitable habitat (i.e. construction of AR) would enhance the natural production of the system. However, at the conference the question was raised if the AR actually do produce more fish or biomass or simply aggregate them at one location (Lindberg, 1997); the attraction-production debate was launched. The outcome of this issue surely has important consequences on the population dynamics of the fishes (Grossman et al., 1997) and on potential management decisions and applications.

We are convinced however, that the attraction-production issue is a simplification of reality. In the continuum of attraction and production an essential part has been neglected so far; namely ecological traps. When an organism is attracted to, and settles preferably in a habitat with suboptimal conditions relative to other available habitats it is attracted to or caught in a so-called ecological trap (Robertson and Hutto, 2006). Habitat choices are a consequence of natural selection and are based upon a number of ecological cues which indicate the quality status of a habitat (Schlaepfer et al., 2002). An ecological trap may occur when changes in the environment act to uncouple the cues used to assess habitat quality from the true quality of the environment (Robertson and Hutto, 2006). It is important to add

the ecological trap to the attraction-production debate as negative ecological consequences for fish may arise from the initial attraction. In the attraction-production issue, the condition of the initial fish stock present might either improve or stay equal; while in the attraction-ecological trap-production issue (as presented here) the situation may also deteriorate.

As proposed by Hixon and Beets (1993) local abundances of fishes are determined by the relative magnitudes of recruitment by larvae, colonization and emigration by juveniles and adults, predation and competition. In addition, fish will allocate their surplus energy from resources to growth and reproduction (Roff, 1983). So, in a simplified model, over time fish will grow and reproduce and mortality (both natural and fisheries induced mortality) will occur. For simplicity, immigration and emigration (Carr and Hixon, 1997) were left out of the model.

If we apply this simplified model to the attraction-ecological trap-production issue three theoretical outcomes are expected (Fig. 1). In the case of attraction, fish' growth, reproduction and mortality in the system observed will be comparable to the reference situation. The carrying capacity of the system does not change. However, spatial dispersion of the fish changes, with aggregation in some places and reduced number in others. If an ecological trap occurs, growth is reduced and/or survival rate is lower compared to the reference situation. Although better alternative habitats are available, the suboptimal habitat is preferably chosen, resulting in reduced carrying capacity of the system. In the case of production, fish have an enhanced growth, a higher survival rate or some combination of both compared to the reference situation, resulting in an increased carrying capacity of the system.

It is important to ask the correct questions to unravel this attraction-ecological trap-production issue (Lindberg, 1997). The outcome is not only influenced by species-specific life history traits, but also by the reef characteristics. The design, the temporal and spatial scale of deployment, location and abundance of reef units all influence fish behaviour (Bohnsack et al., 1994; Carr and Hixon, 1997; Pickering and Whitmarsh, 1997).

Based on the suggestions by Lindberg (1997) and the information by Bohnsack (1989), Brickhill (2005) and Pickering and Whitmarsh (1997) we propose the following questions to unravel the attraction-ecological trap-production issue:

- 1) Does attraction of fish towards the ARs occur?
- 2) If there is attraction of fish, is it age group specific?
- 3) Which mechanisms or processes influence fish production in the ecosystem investigated? Are any of these mechanisms/processes affected by the ARs?
- 4) What is the species-specific behavioural ecology of fish in this ecosystem?
- 5) If there is production of fish, is it sufficient to offset associated fishing mortality?

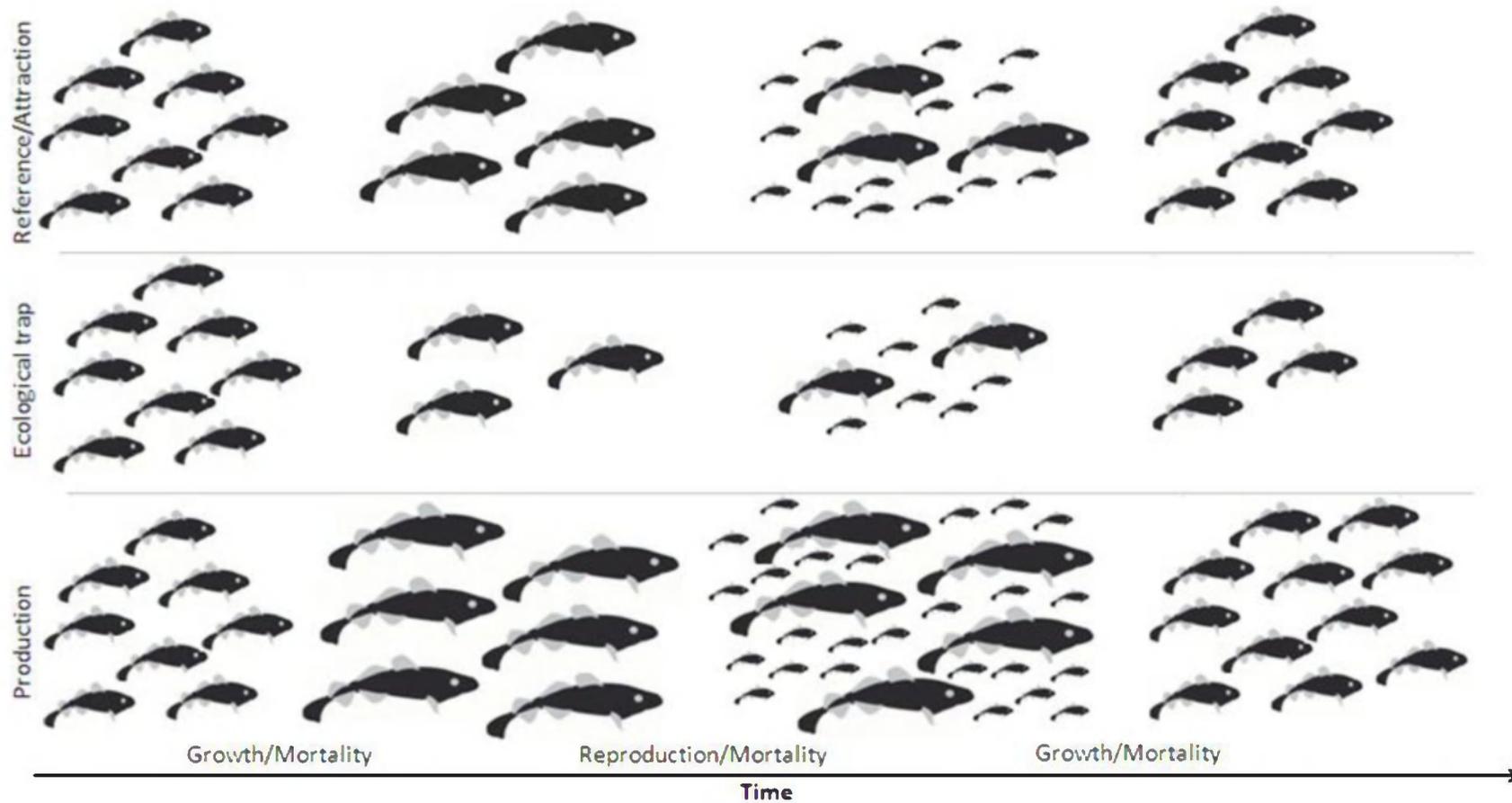


Figure 1. Conceptual representation of the 'Attraction-Ecological trap-Production Issue'

In a reference situation (upper panel) fish grow and reproduce and mortality (both natural and fisheries induced) occurs. If attraction takes place (upper panel), the outcome matches the reference situation, but spatial dispersion differs. In the case of an ecological trap (middle panel), fish have a reduced growth, a lower survival rate or a combination of both compared to the reference situation. If production occurs (lower panel), fish have an enhanced growth, a higher survival rate or a combination of both compared to the reference situation. For simplicity reasons immigration and emigration were left out of the model.

These are not easily answered questions and the mechanisms playing are not always directly observable. The observations should be performed on an appropriate temporal and spatial scale and with many different tools in order to obtain data on several life history characteristics of the fish. The information obtained under questions 3 and 4 should be integrated to understand the mechanisms and behavioural cues playing and to determine the outcome of the attraction-ecological trap-production issue. It is important as well to ask question 2. Fishes can be ‘attracted’ to ARs at two different life stages: as (post)larvae and as older age groups (Wilson et al., 2001). The larvae arrive at the AR through direct settlement after a pelagic phase, while the older age groups arrive through active migration. From the latter, some age groups may be attracted while others aren’t. Question five is highly significant for conservation and management decisions. If the production is not able to offset the fishing mortality, overfishing may occur (Grossman et al., 1997; Rose and Kulka, 1999). The aggregation of fish may increase the access to previously unexploited and exploited stock segments with possible deleterious effects through the increased catch rates.

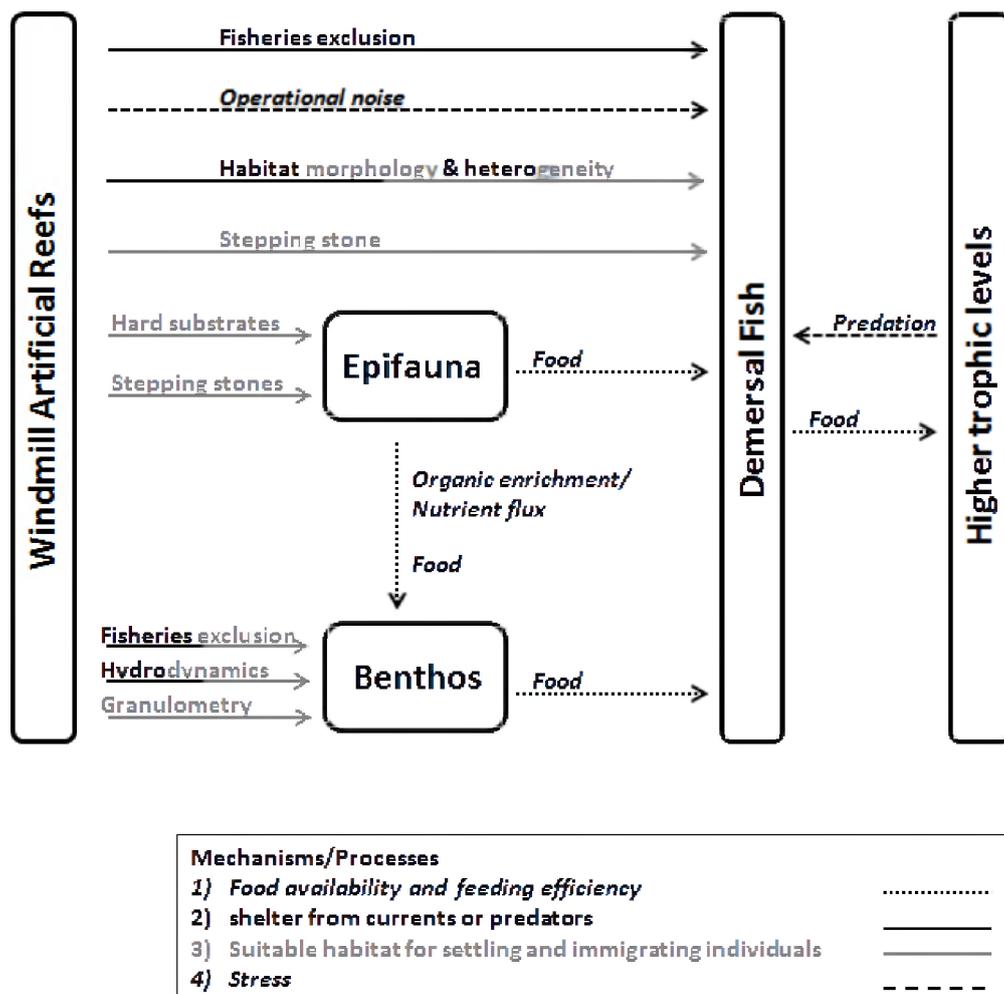


Figure 2. Schematic overview of the most important reef effects influencing fish production at windmill artificial reefs. Each mechanism/process type is indicated by a corresponding line style.

1.3 Artificial reef effects on benthopelagic fishes

Several mechanisms influence fish production at ARs (Bohnsack 1989) by: 1) providing additional food and increasing the feeding efficiency, 2) providing shelter from currents and predators, 3) providing recruitment habitat for settling individuals and suitable habitat for immigrating individuals, 4) causing stress (e.g. noise emission by operational wind turbine, increased predation pressure) (Bohnsack, 1989; Bull and Kendall Jr, 1994; Fabi et al., 2006; Leitao et al., 2007; Randall, 1963). All these mechanisms influence growth, migration, survival and/or reproductive capacity of fishes; thus having indirectly an influence on the reef carrying capacity. In figure 2, a schematic overview of the most important reef effects influencing fish production at WARs is given.

Many studies indicated that fish species may be aggregating at ARs for food. The ARs harbour a wide variety and high abundances of epifaunal organisms (Kerckhof et al., 2010a; Krone et al., 2013; Zintzen et al., 2006), which might serve as a primary food source for reef fishes. Stomach content studies disclosed that many reef associated fish species forage on organisms growing on the reef. However, some species are less dependent of the reef and feed on prey species from the surrounding soft substrates or on a mixture of soft and hard bottom associated prey (Fabi et al., 2006; Leitao et al., 2007; Lindquist et al., 1994; Relini et al., 2002; Reubens et al., 2011).

Shelter possibilities provided by ARs will also affect settlement, early survival rates and post-recruitment interactions (Hixon and Beets, 1989). It is assumed that shelter possibilities are mainly important as protection against predator attacks. Gotceitas et al. (1995) disclosed that juvenile Atlantic cod (*Gadus morhua*) preferred the habitat type providing the best cover when predators were present. If no predators were around, no preference for a specific habitat type was observed. Also in the field, specific predator avoidance behaviour related to substrate type was observed for Atlantic cod (Gregory and Anderson, 1997). Besides, prevailing currents at ARs may influence the aggregation behaviour. If currents are too strong, the reef may provide protection against it. On the other hand, the currents may attract fishes, providing them with food (Jessee et al., 1985).

Marine reserves are promoted worldwide as a tool to protect and conserve fish stocks (Roberts et al., 2001). They have higher average values of density, biomass, organism size and diversity than outside reserves (Halpern and Warner, 2002) and provide a refuge zone where populations of exploited species can recover (Gell and Roberts, 2003). ARs may fulfil the same function as marine reserves and in many offshore wind farms fishery activities are prohibited (Verhaeghe et al., 2011). This safety zone, preventing collision and entanglement of fishing gear, around the wind farms can be seen as a *de facto* marine reserve. In these areas, fish have a chance of higher survival as a result of the released fishing pressure (Langhamer, 2012).

The Southern Bight of the North Sea is dominated by soft substrates and with the introduction of artificial hard substrates, opportunities become available for species that were unable to settle before (Kerckhof et al., 2011). Also for some non-indigenous fish species (range-expanding species) ARs may form a stepping stone.

The reef behavioural ecology of fishes will be influenced by several factors. Some species are reef obligate, while other more opportunistic species will use reefs as well as other habitats (Bohnsack, 1989). Within species, age-specific behavioural preferences may be present as well. Different age groups may occupy different habitats. After the pelagic phase, juveniles from pouting (*Trisopterus luscus*) for instance, settle in estuaries where they stay a couple of months. At the end of their first year, they move to offshore areas (Hamerlynck and Hostens, 1993). Reef availability (both natural and artificial) may influence behaviour too. If a population is limited by reef availability, adding suitable habitat should result in increased abundances. However, populations may also be recruitment limited. Larval survival, dispersal survival or settlement survival may limit the adult populations (Bohnsack, 1989). In this case, adding more suitable habitat to the environment will not result in enhanced abundances. Finally, fish exploitation pressure should be brought into account. Fisheries significantly limit adult (and even juvenile) population levels (Hutchings and Reynolds, 2004), certainly for long-lived species. Commercial fish which are reef dependent may even be more vulnerable to this limitation as they concentrate over hard substrates. This aggregation may increase the access to previously unexploited and exploited stock segments with possible deleterious effects through increased catch rates (Lindberg et al., 2006; Polovina, 1991).

1.4 Research objectives

In the next 10-20 years, thousands of wind turbines will be present in the North Sea. Their foundations form artificial hard substrates (WARs) in an ecosystem that is naturally dominated by soft-bottom sediments. In this paper we investigate the impact of these WARs on the ecology of benthopelagic fish. More specifically we will try to resolve the attraction-ecological trap-production issue for Atlantic cod and pouting at the WARs in the Belgian part of the North Sea.

The objectives of the paper are linked to the questions to resolve the attraction-ecological trap-production issue. In a stepwise approach crucial information on the community structure, behavioural ecology and reef dynamics are acquired to attain an integrated overview of the most important processes steering the occurrence of the fish populations as observed at the WARs.

2. RESEARCH STRATEGY

2.1 Study Area

The research for this work has been performed in the wind farm of C-Power. This wind farm is situated at the Thorntonbank, a natural sand bank in the Belgian part of the North Sea (BPNS) (Fig. 3). The construction works started in 2008 and the wind farm will be fully operational by the end of 2013. Water depth varies between 18 and 24 m and the total surface area of the farm is 18 km².

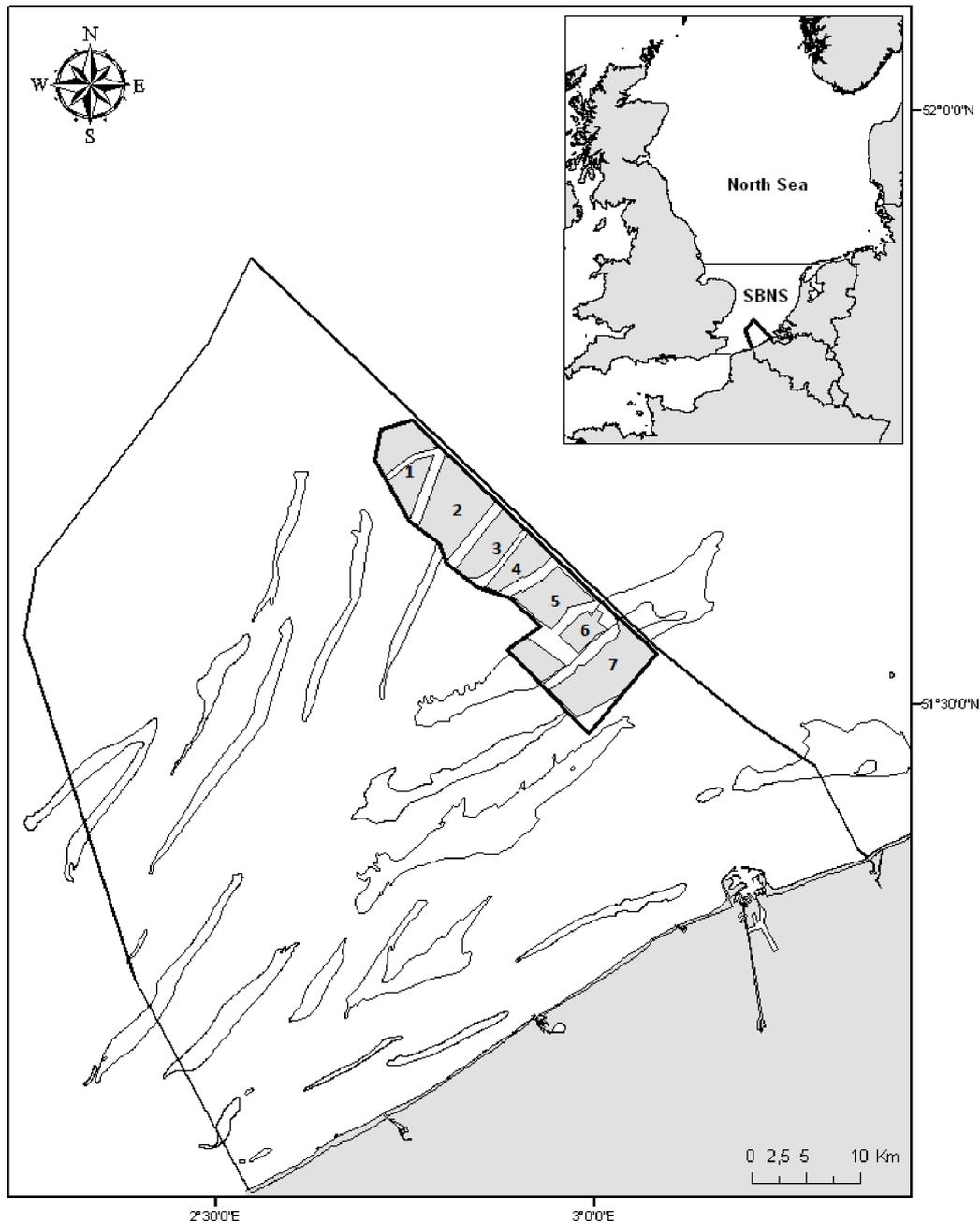


Figure 3. The Belgian part of the North Sea (BPNS) and its position within the Southern Bight of the North Sea (SBNS) and the North Sea. At the East side of the BPNS a zone is dedicated for renewable energy production. All concession areas have been granted for wind power: 1) Mermaid, 2) Belwind, 3) Seastar, 4) Northwind, 5) Rentel, 6) C-Power and 7) Norther. The lines represent subtidal sandbanks.

All samples for this research were taken at gravity based foundations, constructed in 2008 in a pilot phase of 6 wind turbines. These foundations have a diameter of 15 metres at the seabed, at a depth of about 22.5 m at mean low water spring (MLWS). The gravity based foundations are surrounded by a scour protection layer of pebbles and rocks with a maximum radius of 18 m. The total surface area of the hard substrates (turbine foundation and scour protection together) is approximately 2043 m² (Peire et al., 2009). The surrounding soft sediment is composed of medium sand (mean median grain size 374 µm, SE 27 µm) (Reubens et al., 2009).

2.2 Methodology

In this section, a brief overview of methods used is given. Figure 4 summarizes the methods used to unravel the attraction-ecological trap-production issue, linked to the research questions for this holistic approach.

For detailed information on the methodology we refer the reader to the various articles and reports that are available on the specific issues.

Attraction

To investigate whether Atlantic cod and pouting are attracted towards the WARs, catch per unit effort (CPUE) data was gathered from 2009 until 2012 at three habitat types in the BPNS (i.e. WARs, shipwrecks and sand bottoms) and the catch rates were compared. To quantify the CPUE of both species line fishing was performed at all sites.

Visual observations with SCUBA divers were carried out at the WARs between 2009 and 2012. A variation of the stationary sampling method (Bannerot and Bohnsack, 1986) was used to count fishes. All fish species encountered were listed, length and abundance were estimated and behaviour observed.

For further details, please consult Reubens et al. (2013; 2011).

Age groups observed

Length-frequency distributions were built to investigate the age composition in the population. It gives clear information concerning the cohorts present and their length distributions. The younger age groups usually do not have much overlap in length distribution (Daan, 1974), which makes it easier to distinguish the different age groups.

Based on length-frequency distributions the different cohorts of Atlantic cod and pouting present could be clearly separated. Length-at-age keys helped to correctly assign the cohorts to their age group. For details see Reubens et al. (In press-c).

Reef effects influencing behavioural ecology

Food availability and quality

To determine the importance of the WARs as feeding ground for Atlantic cod and pouting, their diet was investigated. In addition, the quality of the food and fitness of the fishes were assessed in relation to specific habitat quality.

The contribution of the prey species present at the WARs was estimated through stomach content analyses and their importance in the diet was assessed through several indices (i.e. frequency of occurrence, numeric and gravimetric abundance index).

Stomach content analyses give valuable information, however they do not render any information on the quality of the prey. Therefore, the proximate composition (proteins, lipids and carbohydrates), the energy content (based on respiratory electron transport system) and fatty acids profiling were estimated for Atlantic cod and pouting and two important prey species: *Jassa herdmani* and *Pisidia longicornis*.

Further, to assess the fitness of Atlantic cod and pouting, length and condition were compared between the WARs and sandy areas in the North Sea (i.e. Goote Bank, BPNS and ICES area IVc). At the WARs and the Goote Bank fishes were collected by standardized line fishing (hooks: Arca size 4; bait: *Arenicola marina*). Samples from the BPNS and the ICES area IVc were collected with beam trawls. All fish were measured for total length and the Fulton's condition index was used as an indicator of the general condition of the fish.

For further details on the food related topics, please consult Reubens et al. (In press-a; 2011; In press-c) and De Troch et al. (Submitted).

Behavioural ecology

In situ observations of behaviour and movements may provide valuable insights in the ecology of fish. However, directly observing the behaviour of marine fish in the wild is logistically very difficult. As a result, other methods are needed to infer fish behaviour. We used acoustic telemetry to investigate residency and site fidelity at the WARs and to empirically quantify movement behaviour of Atlantic cod. Acoustic telemetry allows studying individual behaviour of undisturbed fish for a long period of time.

We tracked 22 Atlantic cod equipped with a Vemco coded V9-1L acoustic transmitter (Vemco Ltd., Halifax, Nova Scotia). Each transmitter has a unique ID, emitting a signal every 110 to 250 s. Fish ranged in size from 28 to 41 cm (total length). The tagged Atlantic cod were tracked with automated acoustic receivers (VR2W from VEMCO). The receivers were placed around two WARs and recorded the presence of any acoustic transmitter within their detection range (i.e. 250 to 500 m). The study ran from May 2011 until July 2012 and some fish were tracked for up to more than a year.

The detection information obtained was used to determine spatio-temporal patterns in presence. Seasonal patterns were investigated in relation to residency and site fidelity at the

WARs, while small-scale spatial patterns were investigated to unravel habitat selectivity of Atlantic cod. For the latter, The Vemco VR2W positioning system (VPS) was used.

For detailed information on the receiver design, position calculation and data analyses performed, please consult Reubens et al. (In press-a; In press-b).

3. DATA OVERVIEW

Attraction

To estimate attraction of Atlantic cod towards WARs, CPUE data were compared between three habitat types in the BPNS: WARs, shipwrecks and sand bottoms. In the first year (2009) after deployment, mean (\pm SD) CPUE at the WARs was much lower (1.2 ± 1.2 ind $h^{-1} fm^{-1}$) compared to subsequent years (5.7 ± 7.4 , 3.7 ± 4.6 and 4.4 ± 3.7 ind $h^{-1} fm^{-1}$ for 2010, 2011 and 2012 respectively). However, from 2010 onwards, cod were clearly attracted towards the WARs. Much higher CPUE values were recorded at the WARs (4.3 ± 6.3) in comparison to the shipwrecks (1 ± 1.2) or sandy bottoms (0.1 ± 0.5). The attraction effect towards the WARs is not the same throughout the year. Significant higher CPUE values were recorded from late spring to late autumn compared to the winter and early spring.

For pouting, attraction towards the WARs was observed from the first year after deployment. Much higher CPUE values were recorded at the WARs (6.6 ± 7.4) in comparison to the other habitat types (0.6 ± 1.1 and 0.4 ± 2.5 for shipwrecks and sandy bottoms respectively). A seasonal trend in catches was present as well, with significant higher values in summer and autumn compared to winter and spring (pair-wise comparisons: $p < 0.05$).

In addition to the catch information, visual observations were performed to estimate densities of fish around the WARs. For pouting reliable observations could be performed, but for Atlantic cod this was not the case. Although present (as confirmed by the catch rates) they were rarely observed by divers. Assessments from visual observations in July-October 2009 revealed an average density of 14 ± 11 individuals/ m^2 of pouting on the scour protection. Further, visual observations revealed seasonality in presence (personal data, unpublished). In winter and (early) spring almost no pouting were encountered during the surveys, while in summer and autumn high abundances were observed (with a peak in late summer).

Age groups observed

The length-frequency distribution data clearly revealed that the I-group of Atlantic cod dominated the catches. They were present year round. Some II-group individuals were caught as well, although in much lower numbers and mainly during the first half of the year. In addition, diver observations revealed the presence of 0-group cod (estimated average length of 5 cm) in late May – early June in 2011 and 2012.

For pouting, both the 0- and I-group were observed at the WARs. The I-group was present year round, though was only well represented from May until October. The first sightings of the 0-group were in August/September.

Mechanisms and behaviour

Food availability and quality

Stomach content analyses on Atlantic cod and pouting gave valuable insights in the diet of both species at the WARs. The gravimetrically dominant prey species in the diet of pouting were *Jassa herdmani*, *Pisidia longicornis*, Pisces spp. and *Liocarcinus* spp. In the diet of Atlantic cod, *J. herdmani*, *P. longicornis*, *Liocarcinus* spp., *Necora puber*, and Pisces spp. were most dominant. Some amphipod species (i.e. *Phthisica marina* and *Monocorophium acherusicum*) had a high frequency of occurrence and reached high abundances, but contributed less to the total prey biomass for both species. All these species are epibenthic organisms abundantly present on hard substrates (samples: Kerckhof et al., 2010a; video observations: MUMM, unpublished data; Kerckhof et al., 2012; Kerckhof et al., 2010b); video observations: MUMM, unpublished data), although some (e.g. *Liocarcinus* spp. and Pisces spp.) may abundantly occur both on hard and soft substrates (personal observations, unpublished).

During visual surveys with divers, feeding pouting have occasionally been observed. Pieces of epifaunal material were bitten off from the stones of the scour protection and ingested (personal observations, unpublished).

Stomach content analyses however, do not give any information concerning the quality of the food. Therefore the energy profile, through proximate composition and fatty acid analysis, of both fish species and some notable prey (i.e. *J. herdmani* and *P. longicornis*) was measured. *J. herdmani* and *P. longicornis* were low in carbohydrate concentrations, but high in proteins. *J. herdmani* was also high in lipid concentrations. Both prey species had a considerable amount of eicosapentaenoic acid (EPA), which is an essential fatty acid which higher organisms cannot synthesize themselves and should gain from their food sources. Atlantic cod and pouting were also low in carbohydrate concentrations, but higher in proteins and lipids. The proteins were mainly stored in the muscle tissue, while lipids were stored in the liver. Both fishes had more energy available than required to maintain the fish metabolism. Thus, enough energy was left for growth and reproduction. As *J. herdmani* and *P. longicornis* have a favourable proximate composition, it is suggested that the WARs form a suitable feeding ground for both Atlantic cod and pouting. However, the proximate composition of other important prey species should be measured too. In this way, a broader view on the suitability of WARs as feeding ground for cod and pouting may be obtained.

If a habitat is suitable as feeding ground for a specific fish species, this should be reflected in the fitness of that fish. A high quality habitat should result in fish with a good fitness. Therefore length and condition (as proxies for fitness) of pouting and Atlantic cod were compared between the WARs and sandy areas in the North Sea. Comparable average lengths were found at the different sites for Atlantic cod. Pouting on the other hand was slightly larger at the WARs compared to the sandy areas, but no significant differences in condition index were observed. The proxies indicate that the fitness of both fish species is comparable at the different sites. For pouting it might even be slightly enhanced. These results demonstrate that for Atlantic cod and pouting the WARs are certainly not inferior in quality to the sandy areas.

Anecdotal observations related to settlement and shelter

As mentioned before, diver observations revealed the presence of juvenile Atlantic cod (0-group) at the WARs in May-June (sampling campaigns of 2011-2012). The individuals had an estimated average length of 5 cm and were observed in low abundances (5 – 20 individuals). The larval and early juvenile stages of Atlantic cod have a pelagic phase of about four months before they settle and become demersal (Cohen et al., 1990). This indicates that the juveniles at the WARs were newly settled individuals.

In addition, the visual surveys gave some information concerning shelter opportunities at the WARs. Several times, Atlantic cod or pouting were observed hiding between rocks of the scour protection. This stone mattress of boulders and rocks, with many holes and crevices creates ideal shelters. The reason the fish were hiding could not be inferred from the observed behaviour. They might seek shelter to protect themselves against currents or predators.

Behaviour of Atlantic cod at WARs - inferred from acoustic telemetry

The results of the acoustic telemetry study showed a strong seasonal variation in occurrence of age-I Atlantic cod at WARs. During summer and autumn fish were present within the study area for an extended period of time and high daily detection rates were observed. By the end of December however, most fish were no longer detected and throughout the winter months (December-March) few detections were encountered. In spring some fish reappeared, although most were not detected anymore at the WARs. This is in agreement with the results from the CPUE data, with catches peaking in summer.

Residency and site fidelity were investigated in closer detail for summer and autumn (i.e. the period cod aggregates at the WARs). Many of the tagged fish occurred at the WARs on a daily basis throughout almost the entire monitoring period, indicating strong residency. Further, high site fidelity was observed as well during this period. The proportion of hour

bins that specimens were detected during their time at liberty was high, with half of the individuals observed at the WARs for more than 75 % of the time.

In addition, the VPS study showed that Atlantic cod are strongly attracted towards the WAR itself (and not to the surrounding habitat for instance). Although the studied area was dominated by soft-bottom sediments and only small patches of hard substrates were available, most of the detections were encountered on the hard substrates or in their close vicinity.

4. DISCUSSION

Between 2009 and 2012 the WARs in the BPNS were intensively sampled to unravel the attraction-ecological trap-production issue for Atlantic cod and pouting. A variety of techniques (catch statistics, telemetry, stomach content analysis, visual observations) were used to collect the necessary data to get insights into the life history characteristics of the fishes.

In figure 4, the results of four years of research are summarized and linked to the research objectives. In summary, it was found that:

- 1) Both Atlantic cod and pouting are attracted towards the WARs.
- 2) More specifically, age groups I & II and 0 & I are attracted for Atlantic cod and pouting respectively.
- 3) There is a seasonality in their presence, they mainly aggregated during summer and autumn
- 4) Both species feed at the WARs, and food has good quality
- 5) Fitness at WARs is comparable or even slightly enhanced compared to other habitats
- 6) Atlantic cod shows high residency and site fidelity

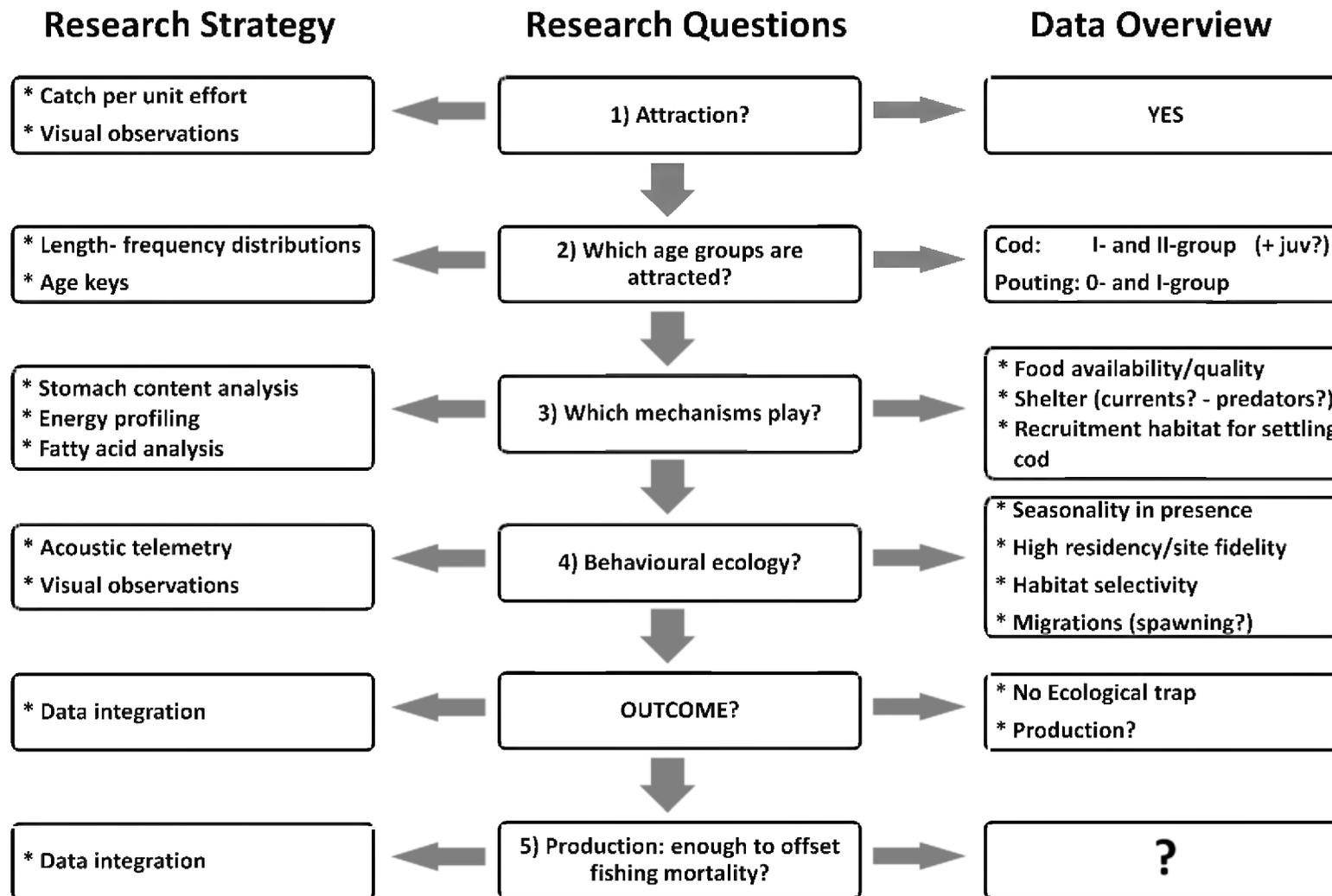


Figure 4. Schematic overview of the holistic approach to unravel the attraction-ecological trap-production issue. In the column on the left the methods used for data analysis are enumerated, in the middle an outline of the research questions is given and in the column on the right the most important results are listed.

The outcome

Based on the integration of our results, we know that Atlantic cod and pouting are attracted towards the WARs and that there is certainly no ecological trap in terms of habitat quality. The fishes feed, grow and have more energy available than required to maintain the basal metabolism. The fitness of Atlantic cod is comparable to the one at the sandy areas. For pouting, fitness is even slightly enhanced at the WARs. For production on the other hand, the situation is more complex. Production cannot easily be measured, which impedes making proper statements.

On a local scale and in terms of extra biomass, one can assume that there is production. Specific age groups are attracted towards the WARs. They show high residency and site fidelity and feed upon the dominant epifaunal prey species present. Growth is observed throughout the period the fishes are present. For Atlantic cod, settling juveniles were observed, thus there might even be production in terms of extra recruitment. In addition, the OWFs in the BPNS are closed to all fisheries activities. As a consequence, fish aggregating in this habitat are released from exploitation pressure; resulting in higher survival chances (i.e. less fishing mortality) (Gell and Roberts, 2003; Polunin and Roberts, 1993). This might lead to an increasing production of the population, although the carrying capacity may be similar to areas outside the OWF.

On a regional scale however, the situation might be different. For example, if a region consists of two local areas and habitat alteration occurred in area A, several scenarios are possible. 1) Nothing changes, fish are not influenced by the habitat alteration; 2) local changes in production occur between area A and B, however the total regional production remains unaltered; 3) local changes in production occur between area A and B, influencing total regional production.

In our situation, no changes in production of Atlantic cod or pouting were observed so far on a regional scale (i.e. BPNS) (Vandendriessche et al., 2012; pers. comm.). Inter-annual variations in catch rates are present, but could not be linked to effects of the OWFs. A multitude of factors; such as environmental conditions, food availability, larval predation, spawning stock structure (Köster et al., 2003; Vallin et al., 1999); influence fish stocks, impeding the assignment of causal relations.

Even though no effects of the OWFs are observed on a regional scale yet, this does not necessarily imply that they are not present. In some cases, the first signs of increased production are observed soon after deployment, while in others it may take many years before changes can be observed or measured (Gell and Roberts, 2003). The time frame within which changes are expected to be measurable depends upon the species investigated, their life-history behaviour and their turnover rate (Pérez-Ruzafa et al., 2008).

In addition, only few WARs were already in place when the research started. In the near future, much more reefs will be present in the BPNS. An upscale in reef numbers may result in an upscale in the area influenced and effects may therefore become more pronounced.

The ecology of Atlantic cod and pouting at the WARs – a summary of life history

Based on literature and on information gathered during this study, a life history reconstruction was made for Atlantic cod (Fig. 5) and pouting (Fig. 6) occurring at the WARs.

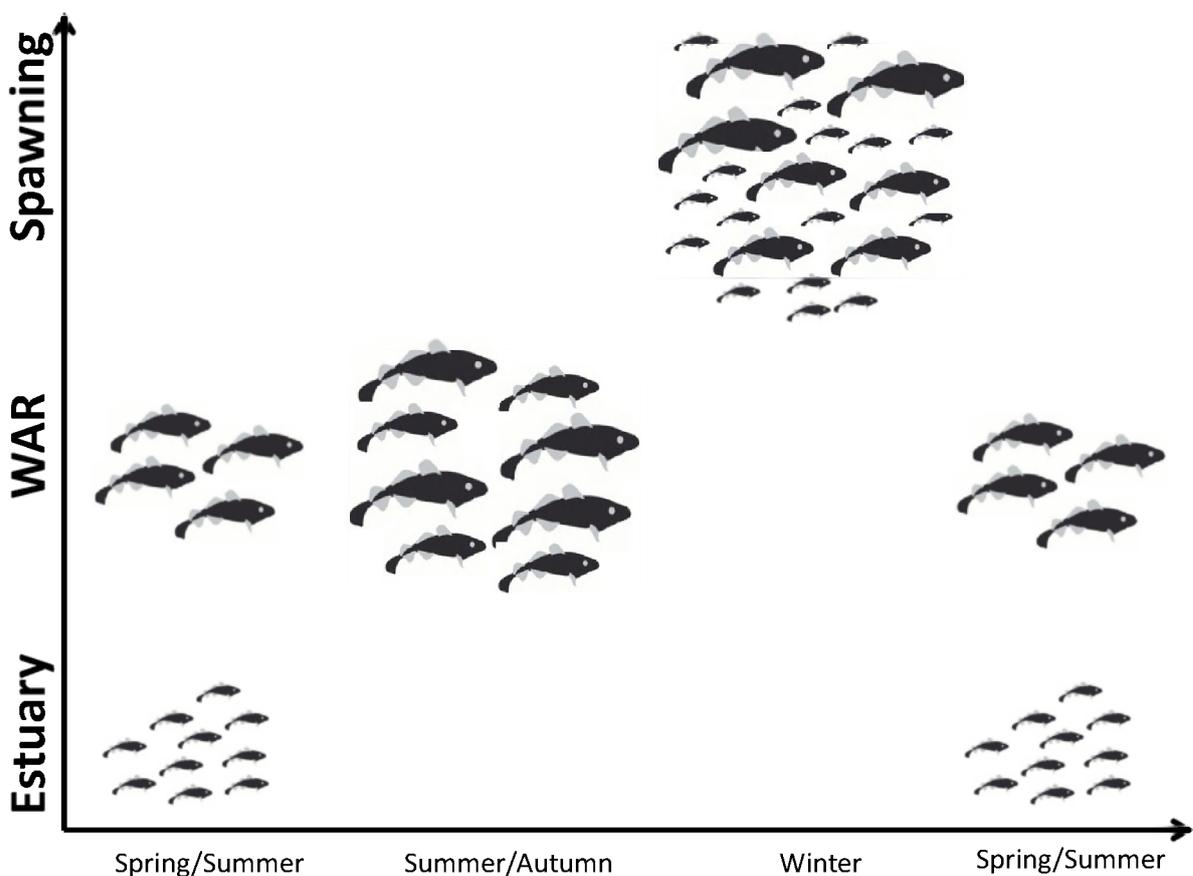


Figure 6. Conceptual representation of the life history of pouting occurring at the WARs. In spring the 0-group lives in estuaries, while the I-group is present at the WARs. Both groups grow and by Autumn the 0-group moved towards the WARs. By winter time they leave the area and move towards the spawning locations (now they became the I- and II-group). After spawning, the newly hatched 0-group pouting arrive at the estuaries while the I-group pouting move back to the WARs. Older age groups probably move elsewhere.

Atlantic cod spawns in winter (December-February) with a peak at the end of January, beginning of February. The spawning areas known in the Southern Bight of the North Sea are in the English Channel and close to the coast of the Netherlands and southeast England (Hutchinson et al., 2001; Righton et al., 2007). The fertilized eggs are pelagic and drift with the currents. After a pelagic phase of four to five months (as egg, larvae and pelagic juvenile) the juveniles become demersal and settle in nursery areas. At that time they reach a length around 5 cm (total length). Although newly settled juveniles were observed at the WARs in May and June, no information is available on the location where the majority of the 0-group juveniles settle. One year later however, around April-May, high abundances of I-group cod are present at the WARs. They stay in this area until the end of the year and growth is observed during this period. By winter time most I-group individuals have left the WARs and probably moved to the spawning areas. Only few individuals come back to the WARs after the spawning period.

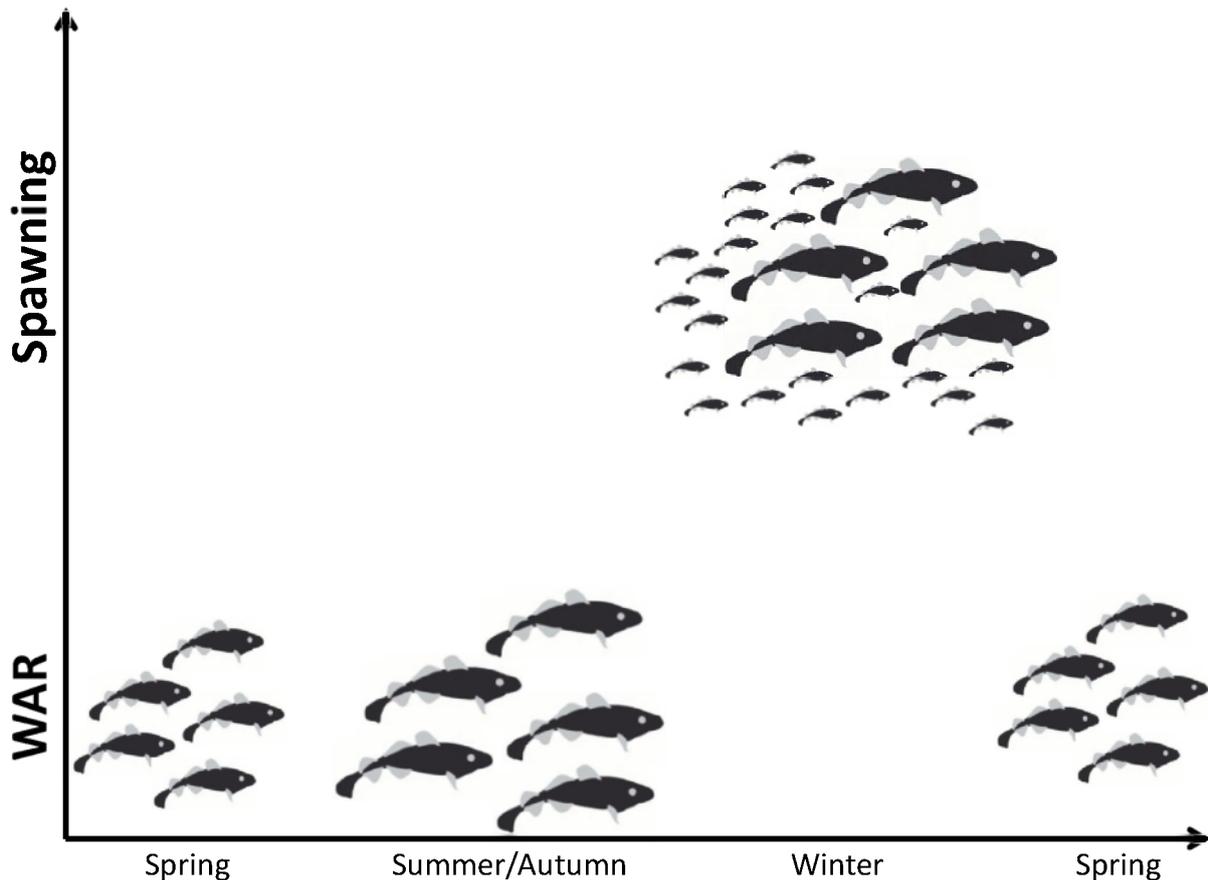


Figure 5. Conceptual representation of life history of Atlantic cod occurring at the WARs. The I-group cod arrive at the WARs in spring and grow throughout the season. By the end of the year they leave the area and move to spawning areas. After spawning most cod do not come back to the WARs. However, one year later the new I-group cod arrive at the WARs. Also some 0-group cod may arrive in spring at the WARs (not shown on the figure).

For pouting, no literature information is available on the spawning locations in our regions. However, it is known that the 0-group pouting live and grow in estuaries and coastal bays. In the Western Scheldt estuary first 0-group arrivals are observed in May, although post-larvae may already be present in April (Hamerlynck and Mees, 1991). The densities peak in June and by October almost all pouting left the estuary. Lengths increase rapidly from less than 8 cm in May to 19 cm in October (total length) (Hamerlynck and Hostens, 1993). The first 0-group pouting arrive at the WARs in September (at a length of 19 cm) and stay till January (became I-group). Most individuals of the I-group have left the area by the end of January however by May they are back at the WARs and stay until the end of the year. Growth is observed throughout this period. The spawning period is from January until April (Merayo, 1996a) and first maturity is at the end of the first year (Cohen et al., 1990); so it is assumed that the I-group pouting move towards their spawning areas in this period, which explains the low abundances at the WARs.

Here, the focus of interest were the WARs. Mind that both species occur at other habitats than the WARs as well (e.g. sandy areas and near shipwrecks).

Allow small-scale passive fisheries?

In the BPNS, all shipping which is not related to the wind farm operations is excluded from the wind farm concession areas (Royal Decree of 11 April 2012, C-2012/11172). As a consequence, all types of fishery activities (except for research) are prohibited in the concession areas. In The Netherlands and Germany the situation is similar to Belgium, while in the United Kingdom fishery activities are allowed inside offshore wind farms. However, restrictions concerning fishing gear, safety perimeter around turbines and length of the vessel are issued and regulations may differ per wind farm (Verhaeghe et al., 2011). On the other hand, pressure groups aiming at the facilitation of passive fisheries inside the wind farm concession areas, are active in Belgium (Verhaeghe et al., 2011).

Whether or not small-scale passive fisheries inside the offshore wind farms would be acceptable from an ecological point of view yet remains an open question. Because the answer to this question has important consequences for management decisions and applications related to fishery activities and ecosystem conservation inside and outside offshore wind farms, we will try to interpret the findings from this PhD study in a passive fisheries context.

In the report of Verhaeghe et al. (2011) an overview of potential fishing techniques inside wind farms is given. Hand line, pot and longline fisheries were evaluated as possible techniques with high potential inside wind farms. However, for fisheries targeting hard substrate associated fish species, only the hand line fishery has potential to be efficient at the WARs. Longlines and pots consist of a mainline with several gangions or pots respectively. The mainline can be hundreds of metres long, while the WARs are just small

patches of hard substrates. Possible target species are sea bass, Atlantic cod, mackerel, pouting and horse mackerel. As the research we performed focused on Atlantic cod and pouting only statements concerning these two species will be made.

Based on the current knowledge on the ecology and population structure of Atlantic cod and pouting at the WARs, we advocate that **no fisheries activities should be allowed inside the OWFs in the BPNS**. We support this statement with four main arguments: s

- 1) The dominance of juvenile fish inside the OWFs
- 2) The seasonality observed in fish presence and abundance
- 3) The scope for OWFs to function as fisheries closures
- 4) Lack of proof of production at the regional scale

Most of the fish present at the WARs are juvenile. For Atlantic cod the age I-group dominates the catches. For pouting it is the 0- and I-group. The European Commission imposes minimum landing sizes for fishes. For Atlantic cod from our region (ICES area IVc) the minimum landing size is 35 cm. For pouting no regulations are set. Atlantic cod at the WARs reach their minimum catch size in the summer of their second year (i.e. I-group). In July, the average size is 34.5 cm, while in September this average is already 37.1 cm (personal data, unpublished). This means that in the first half of the year, the majority of the catch would be below the minimum landing size. Throughout the year, there would be a considerable amount of discard of undersized Atlantic cod (cf. population structuring).

A second argument for fisheries exclusion is the seasonality in presence of fish. As revealed by the telemetry study, most of the cod individuals have left the area by the end of the year. This means the fish leave the protected area once they have reached a proper size. Thus, the population builds up biomass within the reserve (i.e. the wind farm) and exports it beyond the boundaries once they are large enough, benefiting the fisheries industry.

As a third reason for fisheries exclusion, we emphasize the benefits of marine protected areas (MPAs). Currently, the OWFs in the BPNs act as de facto MPAs. Marine reserves (i.e. a type of MPA in which all extraction is prohibited) generally lead to an increase in density, biomass, diversity and individual fish sizes. The effects develop fast and are long lasting (Fenberg, 2012; Gell and Roberts, 2003; Halpern and Warner, 2002; Polunin and Roberts, 1993). If fisheries continue to be prohibited inside the OWF, it would largely benefit local fish stocks. It is expected that reserves would mainly benefit resident species, which are directly protected by the reserve. However, more mobile species may also benefit. Although Atlantic cod for instance has a larger scale of movement than the protected areas, it can still be valuable for specific life stages, offering protection during

vulnerable moments (Gell and Roberts, 2003). In our situation, juvenile Atlantic cod seem to aggregate near the WARs before large scale movements start. During this period they have a higher survival chance through the fisheries cessation. Once they move outside the protected area, they are more vulnerable again. In addition, within cod populations, there are differences in movement characteristics, ranging from residents to active dispersers (Robichaud and Rose, 2004). Resident individuals inside protected areas enable the increase in biomass and reproductive capacity, while the highly mobile animals ensure that the benefits are exported beyond the boundaries of the reserve (Gell and Roberts, 2003). As a result, both fish and fisheries benefit from marine reserves.

So far, there is not yet an indication of production on a regional scale (Vandendriessche, personal communication). Although high catch rates are observed at the WARs, this does not necessarily represent augmented abundances on a region scale. Aggregation of fish may easily lead to overfishing if CPUE data is misinterpreted (Rose and Kulka, 1999). Concentration of fish and fishery may lead to a hyperstability in the CPUE-abundance relationship, leading to an overestimation of the stock size on a regional scale, as only local abundances are represented. As a result unsustainable fishing mortality occurs, leading to overfishing. Further we are convinced that in complex situations where the resilience of the fish population and carrying capacity of the system is not completely understood, the precautionary principle should be endeavoured.

On the other hand, underestimation of stock size on a regional scale can occur as well, in the case of attraction. The ICES International bottom trawling surveys, performed for fish stock assessment in the North Sea, for instance do not monitor inside OWFs. If fish concentrate at the WARs, the stock will be assessed smaller-than-real due to the reduced exploitable proportion of the population within the monitoring area. Therefore, it should be kept in mind that estimated stock size may vary based on the type of assessment performed (i.e. local, small-scale versus region, large-scale monitoring).

In the near future, many OWFs and thousands of WARs will appear in the North Sea. A network of small MPAs (if fishing is excluded from the farms) all along the North Sea will be present, benefiting benthopelagic fish species and fisheries. On the other hand, the increasing number of concession areas will further reduce the available fishing grounds for the fisheries industry. Eventually compensatory measures for fishermen should be taken, if the benefits from the marine reserves seem to be insufficient to compensate the burden generated by the loss of fishing grounds. Proper marine spatial planning, as a tool for sea use management should be implemented, to reduce conflicts and use the marine resources in a sustainable way (Douvere et al., 2007). The ecosystem approach, i.e. the integrated management of habitats and resources used by the fish populations all along

their life cycle (Garcia et al., 2003), should be applied in management decisions to reach both vital fish populations and fisheries activities.

It should be clear that the OWFs are 'areas of opportunity'. Although we support to keep the wind farms as 'fisheries exclusion zones' we do not state that these areas are the most appropriate areas as no-take zones. Other areas in the BPNS (and beyond) may be more beneficial as marine reserves. The designation of marine reserves should be part of the marine spatial planning and should take data from a large region (i.e. the BPNS) and for a multitude of species (marine mammals, fish, birds, benthos) into account (Degraer et al., 2009). This falls outside the scope of this study. The results of this study do however indicate that the OWFs have large potential as areas of opportunity for the fisheries management of Atlantic cod and pouting.

The WARs are a relatively new habitat in the North Sea with ongoing colonization processes and changing dynamics. As a result, the current situation and equilibrium is susceptible to changes. We encourage long term monitoring of the fish stocks (both on local and regional scale) and future research should strive to unravel all factors influencing behaviour and productivity of the stocks. Fish stock dynamics are influenced by ecological processes and human activities throughout the life history of fishes. Therefore, additional information on migration routes and spawning locations should be gathered.

This combination of long term monitoring and additional information on ecological processes influencing fish stock dynamics should allow us to demonstrate whether extra production on population level occurs.

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Chapter 10

Considerations, conclusions and recommendations

The overall aim of this thesis was to unravel the attraction-ecological trap-production issue for Atlantic cod and pouting at offshore wind farms (OWFs) in the Southern North Sea. The outcome of the issue and suggestions for marine management were discussed in chapter 9. In what follows, some findings are questioned in relation to methodological considerations, ecological processes and anthropogenic interactions. Finally, some recommendations for future research on the ecology of benthopelagic fish species at offshore wind farms are formulated.

Species richness of fish is highly dependent on the sampling method

Based on the information obtained from line fishing, on average less than four species were found per sampling event at the three habitats investigated (wind farm, shipwrecks and sandy areas). The average species richness was also somewhat lower in winter and spring compared to summer and autumn (as discussed in chapter 2). Vandendriessche et al. (2011a, 2012) recorded higher species richness (up to 16 species for benthopelagic and demersal fishes combined) at sandy areas both inside and outside wind farms in the BPNS compared to our results. Likewise, the visual observations with scientific divers at the WARs revealed higher species richness than based on the catches by line fishing. For the whole North Sea, 224 fish species have been recorded (Daan et al., 1990). However, the total fish biomass in the North Sea is dominated only by a limited number of species (Kerckhof and Houziaux, 2003).

Line fishing is known to be a selective fishing method and is influenced by type and size of baits, hook design, hook size, fishing strategy and fish ecology (Erzini et al., 1996; Løkkeborg and Bjordal, 1992; McClanahan and Mangi, 2004; Ralston, 1990), which may explain the low species richness observed in our study. As we were mainly interested in the ecology of Atlantic cod and pouting, the selectivity of the fishing gear used, did not influence the results in this thesis and proved to be an appropriate technique. The findings on the community structure (chapter 2) on the other hand should be interpreted with care and it has to be considered as an underestimation of the total species diversity in the area.

Other factors influencing species richness may play a role as well. Diversity at hard substrates for instance may be influenced by the type of substrate. Natural hard substrates are known to be very diverse and may host a more complex and rich community than artificial reefs (Carr and Hixon, 1997; Rooker et al., 1997). Some small zones with outcropping clay beds, gravel beds, pebbles and boulders are present in the

BPNS (Degraer et al., 2006; Kerckhof and Houziaux, 2003; Veenstra, 1969). However, so far the knowledge on natural reefs in our regions is scarce (Van Beneden, 1883).

Fish community structure at the WARs

We demonstrated that specific age groups were present in the catches of Atlantic cod and pouting at the WARs (chapter 8). For Atlantic cod the I-group dominated the catches, while for pouting it was the 0- and I-group. It should be kept in mind that the majority of the Atlantic cod present in the Southern North Sea belong to age group I and II, while older age groups are present in limited numbers (ICES, 2013). It might be that the older age groups are attracted to the WARs as well, but are represented in too low numbers in the Southern North Sea to be observed. This topic merits further investigation.

Although not represented in the catches, other age groups, which are not efficiently sampled, may be present as well at this habitat. Visual surveys with SCUBA divers (Reubens et al., 2011) for instance, observed Atlantic cod as small as 5 cm and pouting of 10 cm at the WARs. As mentioned before, line fishing is a selective fishing technique and type and size of baits, hook design, fishing strategy and fish ecology influence species and size selectivity (Erzini et al., 1996; Løkkeborg and Bjordal, 1992; McClanahan and Mangi, 2004; Ralston, 1990). Prey preferences of fish are related to prey size (Daan, 1973), thus size of bait and hooks will influence the fish size caught. The size of the hook induces a lower and upper limit of fish sizes able to be caught. For small fish the hook used may be too big, while big fish may no longer be efficiently hooked (Løkkeborg and Bjordal, 1992). As a result, gear selectivity might influence the community structure observed at WARs.

However, it might be that the observed catches, resulting from the selected fishing gear, are representative for the true age structure present at the WARs. If this is the case, other mechanisms influence the presence of specific age groups. Specific life history events and life stages influence the presence of fish species at certain habitats. Juvenile Atlantic cod for example are more vulnerable to predation (even by conspecifics) and have other diet requirements than older, mature cod (Daan, 1973). These life history characteristics are related to habitat requirements and should reflect the optimal energy gain. It might be that the WARs are an interesting habitat for juvenile Atlantic cod and pouting, but that older age groups are no longer attracted to this habitat due to changes in their life history behaviour. Predator-prey relationships alter with age, related to prey size preferences (Daan, 1973). Younger Atlantic cod mainly forage on smaller crustaceans (e.g. amphipods, small crabs) which are readily available at the WARs. Older individuals change to a piscivorous diet (Daan, 1973). The complexity of the habitat at the WARs might hamper Atlantic cod to efficiently prey on fish, resulting in suboptimal energy gain for older specimens. In addition, older fish are less vulnerable to predation themselves as cannibalism and predation by other fish species does not longer occur. As a result, older

Atlantic cod are less dependent of protective habitat and hence the complexity of the WARs is no longer beneficial

For younger age classes, fish mortality is mainly determined by predation, while fishery takes over at older age classes (Link et al., 2009). This might be a second mechanism influencing the presence of age groups. Inside the Belgian offshore wind farms no fisheries activities are allowed, enhancing the survival rate of fish present in these areas. Once they left the areas, they are more vulnerable to fisheries (both commercial and recreational). Both Atlantic cod and pouting leave the protective area of the OWFs during winter time, which is probably related to spawning migrations. We do not know where the fish exactly go, but there are indications that it is a journey fraught with peril. Just outside the wind farm concession areas, commercial trawling vessels are frequently operating (Vandendriessche et al., 2011b and personal observations) and fishing pressure is very high. From a field experiment in which Atlantic cod were tagged with an acoustic transmitter, 5 out of the 19 (26 %) tagged fish were returned by recreational fishermen (own observations). Julliard *et al.* (2001) revealed that fisheries mortality of the 0-group Atlantic cod is negligible, but that it is high for older fish. Annually, more than 60 % of the 2 to 4- year-old Atlantic cod in the North Sea are caught by fisheries (ICES, 2013). This indicates that fisheries mortality may influence fish survival considerably and may hence have artificially reduced the probability of fish to return to the wind farm after winter migration.

Standardisation of catch rates at WARs versus shipwrecks

Catch rates (expressed as CPUE) at the shipwrecks were significantly lower compared to the WARs for Atlantic cod and pouting (chapter 4). The lower catches might be an artifact of the sampling procedure. At the WARs the exact location of the reef is visible through the wind turbine foundation and fishing is always feasible at one side of the turbine (in the lee of the current). At the wrecks on the other hand, no surface visual aid is available disclosing their exact position. The research vessel drifts over the wreck, taking into account the prevailing current and wind, and once the ship passed the wreck, it has to reposition to start over again. This implies that one hour fishing at the wrecks is less efficient compared to one hour fishing at the WARs. However it is not possible to account for this in the CPUE, as strength of the currents and wind speed and direction, which are highly variable, influence the fishing time. In some occasions the ship was correctly positioned for long time, while in others (strong wind and current from the same direction) positioning was more difficult.

However, even if this is taken into account, it cannot fully explain the differences in catch rates observed between the two types of reefs, so other mechanisms contribute to the observed differences in aggregation of fish. Several studies have shown that artificial

reefs often have a higher density, biomass and diversity compared to natural reefs or to older artificial reefs (Ambrose and Swarbrick, 1989; Bohnsack et al., 1994; Leitao et al., 2008; Rilov and Benayahu, 2000; Walsh, 1985). Prey availability, predation pressure, habitat complexity and refuge possibilities, reef size and reef isolation were proposed in the above mentioned studies as factors possibly influencing the establishment of fish assemblages. Also the vertical relief of wind turbines may explain higher species richness. The relief creates niches for fish species from various depths and allows high recruitment rates (Pickering and Whitmarsh, 1997; Rilov and Benayahu, 2000).

Another factor that has not been taken into account thus far is the impact of fisheries activities near the reefs. If human disturbance at reefs through recreational and commercial fishing is considerable, this may seriously influence the fish community structure present (Roberts, 1995). Fishery activities may lead to a decrease in diversity, density and fish size (Currie et al., 2012; Jennings et al., 1995; McClanahan and Muthiga, 1988), while marine reserves have the opposite effect. In the BPNS shipwrecks are often targeted by recreational fishermen (hook and line fisheries) and even commercial trawling vessels fish as close as possible to shipwrecks, because of the aggregated fish densities (personal communications). The OWFs on the other hand are closed to all types of fisheries (see chapter 9). The difference in fishing pressure between the two types of reef may partly explain the differences in abundances observed. To our knowledge, no research has been conducted on this issue yet in our regions.

Wind farms closed to fisheries

In chapter 9 some arguments were given to explain why wind farms should be closed to fisheries. These arguments are based on the knowledge gathered in this PhD study and have an ecological viewpoint. In addition, there are some more arguments; from a social, economical and political point of view to retain the wind farms closed to fisheries.

A major concern from the concessionaires is the risk of collision. A collision can result in a lot of damage to the vessel and the wind turbine and may have a large environmental impact, resulting in huge economical and financial drawbacks (van Iperen and van der Tak, 2009). If no fisheries are allowed, collision risks are significantly reduced.

Secondly, the policy aims to increase the biological valuation of the wind farm concession areas. An action plan (Actieplan Zeehond) has been introduced to promote biodiversity in the region (Vande Lanotte et al., 2012). Reef balls will be deployed in summer 2013 in two concession areas in the BPNS (Rabaut, personal communication). These environmental measures do not go along with fishery activities in the same area.

Mariculture and specifically sea ranching were not mentioned in this study. Sea ranching is defined as 'the release of cultured juveniles into unenclosed marine and estuarine environments for harvest at a larger size' (Bell et al., 2008) and has great potential in

aquaculture (Zion and Barki, 2012). However, assessing the environmental impact of sea ranching falls out of the scope of this study and no statements about the possibilities of sea ranching in combination with a sustainable management of the 'native' fish community at the WARs can be made.

The Bigger picture – thinking outside the box

The behavioural ecology of Atlantic cod and pouting at the WARs and the driving mechanisms influencing this behaviour are now documented. We know the time frame both fish species are present and which age groups are aggregating. However, information on migration routes, spawning locations and population of origin of both species is still scarce. Ecosystem-based fisheries management, taking ecosystem components and services into account in managing fisheries (Garcia et al., 2003), requires detailed information upon habitats and resources used by the fish populations on a temporal and regional scale.

Atlantic cod is known to make extensive migrations from feeding (i.e. in summer and autumn) to spawning grounds (i.e. in winter time) (Turner et al., 2002) and Righton et al. (2007) showed that stocks from the Southern North Sea have spawning areas along the Southeast coasts of the United Kingdom, the coasts of the Netherlands and in the English Channel. During summer mixing of the stocks may occur in the Southern and Central North Sea, although influx of the English Channel is expected to be limited (Righton et al., 2007). Information concerning the population sources of Atlantic cod present in our regions during summer and clues about potential homing behaviour are missing. For pouting, no information is available on the spawning locations and population characterisation. The post-larvae and juveniles grow up in estuaries (Hamerlynck and Hostens, 1993).

The results from this thesis cannot be seen isolated from the bigger picture of offshore wind energy developments. The insights regarding reef effects playing for benthopelagic fish indicate that Atlantic cod and pouting will benefit, at least on a local scale, from the OWFs. However, cumulative effects on a larger scale are difficult to predict. We assume that the extension of OWFs over a large area may have both positive and negative effects for fish populations. Some personal considerations:

- 1) The many wind farm concession areas granted will result in a lot of areas being closed for fisheries. In these areas local fish stocks will benefit from the released fishing pressure. On the other hand, areas open to fisheries may experience intensified fishing activities, due to the reduction in available fishing grounds.
- 2) Marine spatial planning may, indirectly, be influenced by the renewable energy development. Extensive zones, dedicated to renewable energy concessions, will be closed

to all kind of activities and thus act as *de facto* marine protected areas. Although these areas are 'areas of opportunity', they might then be indicated as marine reserves, as they are already present. However, these areas are not necessarily the most appropriate areas for protection and conservation. This might result in biodiversity losses and reduced carrying capacity of the ecosystem as not the most appropriate habitats are protected.

3) Non-indigenous fish species might benefit from the WARs and use them as stepping stones to colonize new habitats.

4) Local fish stocks may benefit from the increased food availability from the epifaunal communities present at the WARs. Certainly the vertical relief of the wind turbines increases, on a limited area, the available habitat for epifaunal colonization and thus the available food for fish.

5) Results cannot directly be extrapolated from one area to another. Results from this thesis do not indicate negative effects of OWFs on benthopelagic fishes. But fish populations at OWFs in other regions may react differently to the changes in their environment. If an OWF would for instance be built at a meeting point or spawning ground, it could possibly have a reverse effect on aggregation/spawning behaviour. To our knowledge, no studies comparing effects on a larger spatial scale have been performed hitherto.

General conclusions

The integrated approach, combining several sampling techniques to investigate the ecology of benthopelagic fishes at windmill artificial reefs, greatly contributed to the understanding of the mechanisms and processes playing at this habitat.

Both Atlantic cod and pouting are attracted towards the WARs. High abundances of the species are observed near the artificial hard substrates and show a seasonal pattern in presence. Aggregation mainly occurs during summer and autumn, while in winter and early spring much lower numbers of fish are encountered. Analysing the community structure in closer detail revealed the presence of specific age groups of Atlantic cod and pouting near the WARs. Not all age groups of a species are necessarily attracted to the same environment. Specific life stages need specific habitat characteristics, which may alter with age. The WARs seem to be interesting to younger life stages as age groups I & II and 0 & I are attracted for Atlantic cod and pouting respectively. Mind that the majority of the Atlantic cod present in the Southern North Sea belong to age group 2 or below (ICES, 2013).

Through acoustic telemetry relevant information on the movement behaviour of Atlantic cod inside an offshore wind farm was gathered. The Atlantic cod present at the WARs (i.e. age group I) are strongly resident and have a high site fidelity throughout the summer and autumn. In winter they leave the protective habitat of the wind farm and move towards

other areas. This migration is probably related to spawning events, but more information is needed to confirm this hypothesis. On a smaller temporal scale, Atlantic cod at the WARs exhibits crepuscular movement patterns, related to feeding activity. Close to sunrise and sunset the highest peaks in detection are encountered.

Several reef effects contribute in the attraction of Atlantic cod and pouting towards the WARs. Availability of food and feeding efficiency certainly plays an important role in attractive power. Food is plentiful and readily available at the WARs (Kerckhof et al., 2010a), increasing the feeding efficiency and reducing the need for extensive movements to find sufficient prey to remain in good condition. Diet analyses confirmed that Atlantic cod and pouting mainly feed upon the dominant epifaunal prey species present (Reubens et al., In press-a; Reubens et al., 2011). Other reef effects may attribute to the observed attraction as well. The WARs have high heterogeneity, providing the fish with opportunities to seek for shelter if needed (for instance in case of strong currents or predator attacks).

Fitness of the fish species present can provide useful information in relation to habitat quality. Attraction towards a specific habitat does not necessarily imply that the habitat has proper quality. Fish may also be caught in a so-called ecological trap, when they are attracted to and preferably settle in a habitat with suboptimal conditions relative to other available habitats (Robertson and Hutto, 2006). Length, condition and diet composition of the fish can be used as proxy to investigate their fitness. Based on these proxies, we found evidence that the WARs do not act as ecological trap for Atlantic cod and pouting.

In conclusion, we demonstrated that windmill artificial reefs influence the behavioural ecology of Atlantic cod and pouting. They benefit from these artificial hard substrates and thrive well in this environment closed to fisheries. We support this fisheries closure and believe that the benefits are exported beyond the boundaries of the wind farm concession, as the fish leave the protective area once they grow older. We stress that the OWFs are 'areas of opportunity' and are not necessarily the most appropriate areas as marine reserves. Proper management, through well-thought-out marine spatial planning and regulations, should be implemented to reduce conflicts and use the marine resources in a sustainable way.

Recommendations for further research

Within the framework of this PhD work some restrictions of the study were notified. Through trial and error some suggestions could be made for future sampling and with insights gained and questions answered, new ones were raised. In what follows, some recommendations for further research are made and new research questions are launched.

- Acoustic telemetry has proven to be a valuable and feasible tool for the study of behavioural fish ecology. Important information concerning behavioural patterns of Atlantic cod was obtained. This information integrated with other data on abundances, biomass and age composition opens possibilities to answer broader ecological questions. In future research the use of acoustic telemetry should be expanded. This fast evolving technology has a multitude of options to gather extensive amounts of data on physiological parameters of the fish and the environment. Valuable insights into the life cycle of the species of interest can be gained. Especially light-based geolocating archival tags have a high potential to be used to investigate fish migrations on a larger scale.
- Every type of fishing gear has a species-specific selectivity. The use of different fishing techniques will reflect the true community composition. For community composition estimates at the WARs, we suggest to combine line fishing with the placement of gill nets and visual observations.
- Scientific diving should be recognized as a valuable sampling tool in temperate waters and be implemented more in the sampling strategy in further research. Sampling with scientific divers allows *in situ* observation of the ecosystem investigated. Advantages of standardized diving observations are (1) sampling in habitats previously unable to attain (e.g. setting gill nets on the scour protection of wind turbines), (2) the positioning of the sample location (e.g. macrobenthos sampling at the edge of the scour protection) and (3) underwater visual observations (I had 44 hours of diving so far near the WARs) gave me a more complete view and insights of what is happening at this habitat (no longer 'blind' sampling).
- In this thesis the WARs were compared with a hard substrate (i.e. shipwrecks) and a soft substrate (i.e. sandy areas) control. Some natural hard substrates (e.g. pebble and boulder reefs) are present in the BPNS as well. However, the structural and functional diversity of this biotope is poorly known (Houziaux et al., 2008) and

the localization of these reefs are not clearly documented. For future impact assessments of the WARs, the ecological characteristics of the natural reefs would be useful to assess the importance and value of the faunal diversity at the WARs.

- In this study, gravity based foundations (GBFs) were investigated as this was the only type present in the BPNS when the research started. Recently, monopiles and jacket foundations are implemented at several OWFs. Differences in reef effects are expected for the different foundation types. Jackets for instance, usually don't have a scour protection layer at the bottom. Varying hydrodynamic conditions and different fish behaviour are expected in comparison to the GBFs. Monopiles consist of steel, having different settlement characteristics for epifauna in comparison to the concrete foundation of GBFs; in addition do monopiles normally have a smaller scour protection.
- Age structure is a relevant indicator for of the health of fish stocks (Probst et al., 2013). In this thesis, age structure was analysed based on length frequency measurements. Only from the last catches, otolith information from a small number of individuals is available, but too few to include the data in our analysis. We know otoliths readings are the most reliable way to determine the age structure within a fish population. Hence, emphasis on otolith investigations should be given in the future.
- The behavioural ecology of Atlantic cod and pouting and the driving mechanisms influencing this behaviour are now documented, but information on migration routes and spawning locations of both species is still scarce. The combination of genetic analyses (through SNP markers) and acoustic telemetry (using geolocating archival tags) would largely contribute to gain insights into the migratory behaviour. Knowledge on the entire life history cycle of fish and their habitat use and distribution range is crucial for optimal management and conservation issues.
- Fisheries activities may lead to a decrease in diversity, density and fish size (Currie et al., 2012; Jennings et al., 1995; McClanahan and Muthiga, 1988), while marine reserves have the opposite effect. In the BPNS shipwrecks are often targeted by recreational fishermen (hook and line fisheries) and even commercial trawling vessels fish as close as possible to shipwrecks, because the aggregated fish densities present are an interesting target (personal communications). The OWFs on the other hand are closed to all types of fisheries (see chapter 9). The difference in fishing pressure between the two types of reef may explain the

differences in abundances observed. To our knowledge, no targeted research has been conducted on this issue yet.

- Last but not least, a question that intrigued me during the past years: “Do Atlantic cod spawn in the Belgian Part of the North Sea?” So far, no scientific evidence confirms the presence of spawning aggregations in our waters and no eggs or larvae of Atlantic cod were observed in water samples. However, they seem to move towards the entrance of the Western Scheldt estuary in winter time and adult specimens with ripe gonads are caught by fishermen in coastal areas during spawning season (personal communications).

Addenda

Addendum 1

Survival of pouting from acoustic tagging

Adapted from:

Reubens J, Delbare D, Degraer S, Vincx M, 2012. The effect of a dummy acoustic transmitter insertion on the survival of pouting, *Trisopterus luscus* L. Belgian Journal of Zoology 142: 130-132

Acoustic fish telemetry is an often used technology that can provide valuable data on fish movement, behaviour and habitat use. In recent years, many novel applications and ameliorated transmitter designs made it an increasingly popular tool in fisheries research (Cooke et al., 2004; Heupel et al., 2006a; Meyer et al., 2007; Righton et al., 2006), resulting in a substantially improved knowledge on behavioural, ecological and physical issues (Abecasis et al., 2009; Bellquist et al., 2008; Cooke et al., 2011; Espinoza et al., 2011; Heupel et al., 2006b) of many fish species in previously out of reach environments. Heupel et al. (2006a) stated “any aquatic species to which a transmitter can be attached or implanted without modifying the behaviour of the animal is potentially suited to this technology”. As a rule of thumb the size of the transmitter and the disturbance to a fish should be minimized in order to study the fish behaviour (Jepsen et al., 2005).

Monitoring of fish communities in wind farms in the Belgian part of the North Sea (BPNS) revealed that pouting, *Trisopterus luscus* (Linnaeus, 1758), was present in high densities in the vicinity of the wind turbines during parts of the year. There is evidence that the food availability for pouting increased at these wind turbines (Reubens et al., 2011). To study the spatio-temporal migration and site fidelity of pouting at the offshore wind farms, acoustic telemetry is planned to be used. However, pouting is a very sensitive species that survives manipulations only in very low percentages (personal observations). As survival rates are indispensable to assess the tagging experiment’s likelihood to succeed, a laboratory experiment was set up to investigate the potential of pouting to be used in acoustic telemetry studies. Consequently, the outcome of our laboratory experiment could be valuable for future applications. To our knowledge, this is the first experimental study in acoustic telemetry on pouting.

The pouting used in the experiment were collected at a wind farm in the BPNS, using hook and line gear. After capture the fish were kept in an aerated water tank for transportation to the aquarium facilities (water temperature of 14°C) at the Institute for Agricultural and Fisheries Research. After a acclimatisation period of 5 to 7 days, the fish were starved for two days (Pedersen and Andersen, 1985) before the surgical operation, in order to maximize the intestinal space for tag insertion. Surgical procedures were similar to those of Baras &

Jeandrain (1998), Arendt et al. (2001) and Jadot et al. (2006). Prior to tagging the fish were anaesthetized in a 0.3ml l⁻¹ 2-phenoxyethanol solution. Following anaesthesia, showing no reaction to external stimuli, slow opercular rate and loss of equilibrium (McFarland and Klontz, 1969), the fish were placed ventral side up in a V-shaped support. Most of the body, except the ventral side, stayed in the water and a continuous flow of aerated water was pumped over the gills to avoid gill damage and to provide a continuous oxygen supply (Taylor et al., 2011). A small incision (15-22 mm) was made on the mid-ventral line and a dummy acoustic transmitter (Vemco, coded, V9-1L) was inserted in the visceral cavity. The incision was closed with two sutures (polyamide monofilament, DS19 3/0). All instruments and transmitters used were disinfected with iso-betadine®. In total, 15 specimens were tagged with a dummy transmitter. The control group, of 10 specimens, were anaesthetised to mimic the handling procedure.

After the surgical procedures, all pouting were stocked together in a fish tank (2 x 2 x 0.5m³) on recirculation (i.e a closed system in which no extra water is added). The tank was checked daily for survival and tag retention. Pouting were fed with fish fillets. The experiment ran for six weeks.

Fish survival rates were compared using chi-square tests. A Two-way contingency table was constructed for survival (dead-alive)/treatment (tag-control) comparison. Statistical analysis was performed in R (version 2.5.1n www.r-project.org). T-tests on the difference in total length of pouting between the treatments were carried out in Statistica (version 7.0, Statsoft, Tulsa, Oklahoma). A significance level of $p < 0.05$ was used in the tests. Results are expressed as mean \pm SD.

The fish length varied between 14.5 cm and 27.5 cm and between 17.3 cm and 28.5 cm for the tagged and control group respectively. No significant differences in length were present between both groups (T-test, $p = 0.49$). In the first week after surgery a significant difference in survival rate (χ^2 -test, $p = 0.041$) was detected between the tagged group (survival: 66.7 %) and the control group (survival: 100 %). Data screening showed that there was a tendency in survival towards larger fish within the tagged group. The fish that died had an average length of 20.5 ± 3.5 cm, while the fish that survived had an average length of 23.2 ± 4.2 cm. However, no significant differences in length were present between both groups (T-test, $p = 0.24$). From the second week onwards there was no more mortality in either of the groups. However, one tagged fish expelled its transmitter in the third week. During the whole period of the experiment all fish ate well and a small increase in length was observed. In the tagged group, overall average length increased from 22.8 ± 4.3 cm to 23.2 ± 4.2 cm, while in the control group it increased from 23.0 ± 4.2 cm to 23.2 ± 3.9 cm. Individual length increment was not monitored as several individuals lost their external identification tag during the experiment. Only fish that survived the experiment were used to calculate average lengths.

The experiment took place in the run-up to the spawning season and post-mortem investigation revealed that some specimens had maturing gonads.

Although the experiment being small-scale (due to limited number of pouting that could be caught), some clear trends were disclosed. A significant lower survival chance was present for tagged pouting in comparison with non-tagged pouting. The results suggest that survival may be influenced by length. Larger animals tend to have higher survival chances, compared to smaller specimens. The experiment clearly showed that if tagged animals died, it was within the first week after surgical procedures. Therefore, it is suggested that pouting does have the potential to be used in telemetry experiments.

However, as survival is indispensable to maximise a tagging experiment's likelihood to succeed, it should be assured that only animals in good condition are released. Therefore, an observation period of one week after surgical procedures is essential to monitor the condition of the fish and to allow them to recovery from stressors (Oldenburg et al.). In addition, specimen above a minimum length should be used. It is suggested to use fish of at least 23 cm, which is the average length of the tagged fish that survived. Based on the facts that all pouting in captivity ate well, increased slightly in length and gonads matured, it is suggested that tagging did not influence their growth and feeding behaviour.

Addendum 2

Details on the fishing gear used

Line fishing is known to be a selective fishing method and is influenced by type and size of bait, hook design, hook size, fishing strategy and fish ecology (Erzini et al., 1996; Løkkeborg and Bjordal, 1992; McClanahan and Mangi, 2004; Ralston, 1990). The gear used will thus largely determine which species will be caught. Therefore, the details of the terminal tackle used in this study are given (Fig. 1).

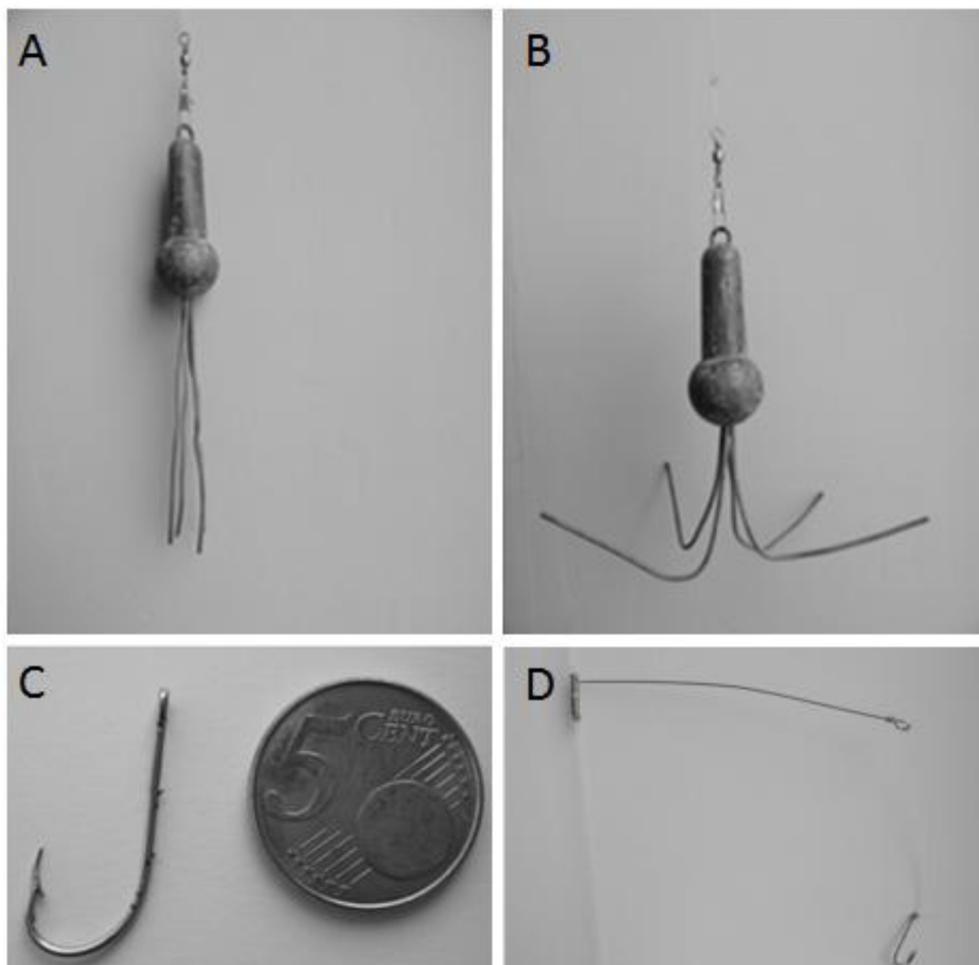


Figure 1. Terminal tackle used for line fishing. A) sinker with closed anchor; B) sinker with opened anchor; C) Hook; D) baitholder.

Terminal tackle: “Surf leader baitholder” with three hooks

Hooks: “Arca” brand, size number 4

Sinker: at the end of the line a sinker of 200 to 250 g was attached.

The sinker had anchors. At the wrecks and the WARs the anchors were closed to avoid getting stuck on the hard substrates. The anchors were opened when fishing was performed at the sandy areas.

Bait: lugworm (*Arenicola marina*)

Fishing technique: For fishing, the terminal tackle was positioned just above or on the seabed.

Addendum 3

Receiver mooring

In acoustic telemetry, a proper receiver mooring device is an important parameter for the study to succeed. The mooring should stay in place and should allow easy recovery of the receivers. In addition, an appropriate method for easy (surface) relocation of the mooring device is needed; certainly in North Sea waters, where underwater visibility is limited.

In figure 1 the mooring device used in the current study is shown. The receivers were deployed near the seabed using a cast iron heating element as mooring. The mooring had a weight (in air) of approximately 80 kg and was supposed to stay in a fixed position. The receivers were attached to a polypropylene rope (using tie-wraps) with the hydrophones in upward position. An underwater buoy was attached to the rope, approximately one metre above the receiver. The rope was connected to the surface with a surface buoy.

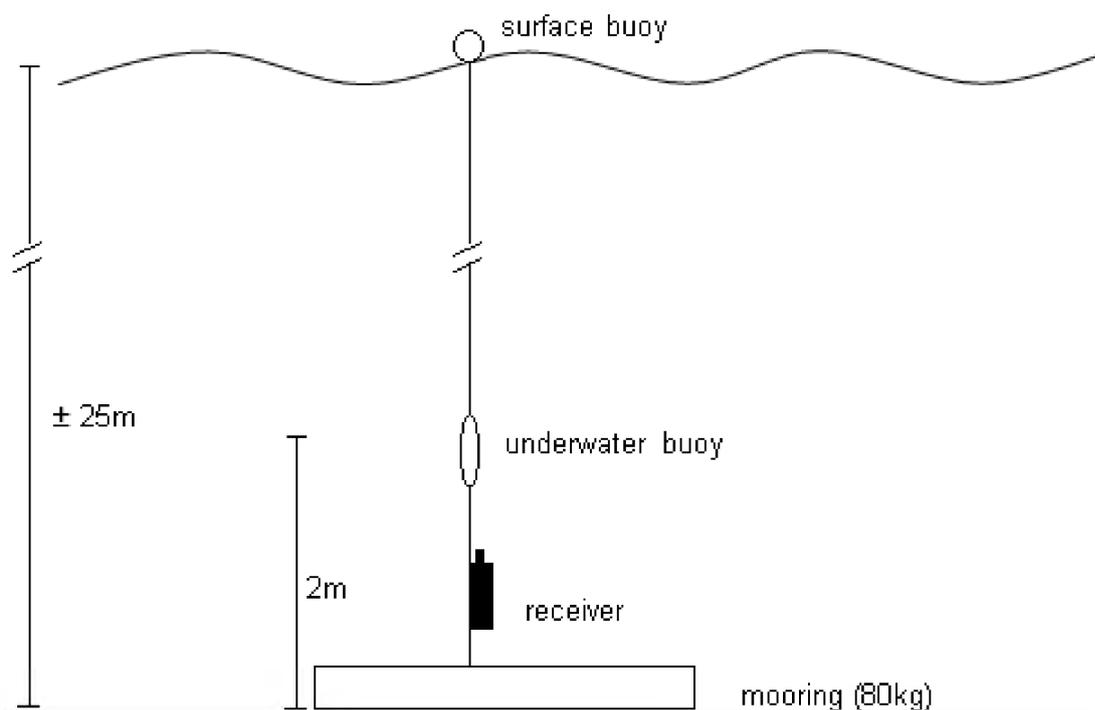


Figure 1. Receiver mooring scheme used in the present study.

The experience obtained from the current study revealed that the mooring itself was very useful. The cast iron heating elements did not move by the currents and receivers were easily recovered (by cutting the tie-wraps). The surface and underwater buoys however caused more problems. Due to the currents the underwater buoy was pushed down in

periods of strong currents, reducing the detection capability of the receivers. Some of the surface buoys on the other hand were stolen, removed by wave action or driven over by vessels. In addition, strong fouling of mussels reduced lifting capacity of some of the bouys.

Therefore, we recommend:

- to substitute the polypropylene rope between the mooring and the underwater buoy for a stainless steel pole. This will prevent the receiver being pushed towards the bottom. As a result detection capability should remain the same in all current conditions.
- to connect the mooring to a (semi)permanent structure (e.g. cardinal buoy, wind turbine) instead of using surface buoys to relocate the mooring device.
- the use of cast iron heating elements as mooring.
- regular data upload and cleaning of the mooring devices to prevent excessive fouling.

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