

University of Groningen

Impact of an artificial structure on the benthic community composition in the southern North Sea

Klunder, Lise; Lavaleye, Marc S.S.; Filippidi, Amalia; Van Bleijswijk, Judith D.L.; Reichart, Gert Jan; Van Der Veer, Henk W.; Duineveld, Gerard C.A.; Mienis, Furu

Published in:
ICES Journal of Marine Science

DOI:
[10.1093/icesjms/fsy114](https://doi.org/10.1093/icesjms/fsy114)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Klunder, L., Lavaleye, M. S. S., Filippidi, A., Van Bleijswijk, J. D. L., Reichart, G. J., Van Der Veer, H. W., Duineveld, G. C. A., & Mienis, F. (2020). Impact of an artificial structure on the benthic community composition in the southern North Sea: Assessed by a morphological and molecular approach. *ICES Journal of Marine Science*, 77(3), 1167-1177. <https://doi.org/10.1093/icesjms/fsy114>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Contribution to the Themed Section: 'Decommissioned offshore man-made installations' Original Article

Impact of an artificial structure on the benthic community composition in the southern North Sea: assessed by a morphological and molecular approach

Lise Klunder^{1,2*}, Marc S. S. Lavaleye³, Amalia Filippidi³, Judith D. L. van Bleijswijk⁴,
Gert-Jan Reichart^{3,5}, Henk W. van der Veer¹, Gerard C. A. Duineveld³, and Furu Mienis³

¹Department of Coastal Systems, Royal Netherlands Institute for Sea Research and Utrecht University, PO Box 59, 1790 AB Den Burg Texel, The Netherlands

²Marine Evolution and Conservation, Groningen Institute of Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

³Department of Ocean Systems, Royal Netherlands Institute for Sea Research and Utrecht University, PO Box 59, 1790 AB Den Burg Texel, The Netherlands

⁴Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research and Utrecht University, PO Box 59, 1790 AB Den Burg Texel, The Netherlands

⁵Faculty of Geosciences, Utrecht University, Princetonlaan 8A 3584 CB, Utrecht, The Netherlands

*Corresponding author: tel: + 31 (0) 222369476; fax: + 31 (0) 222319674; e-mail: lise.klunder@nioz.nl.

Klunder, L., Lavaleye, M. S. S., Filippidi, A., van Bleijswijk, J. D. L., Reichart, Gert-J., van der Veer, H. W., Duineveld, G. C. A., and Mienis, F. Impact of an artificial structure on the benthic community composition in the southern North Sea: assessed by a morphological and molecular approach. – ICES Journal of Marine Science, 77: 1167–1177.

Received 1 April 2018; revised 27 July 2018; accepted 9 August 2018; advance access publication 12 September 2018.

Man-made structures in the North Sea are known to act as artificial reefs by providing a habitat for sessile epifauna in a predominantly soft sediment environment. This epifauna is hypothesized to cast a so-called “shadow” over the soft sediment ecosystem by altering the nutrient composition in the overlying water column. In addition, the structure itself could alter currents and thereby influence the deposition and erosion of the sediments in the wake of the platform. This study aims to assess the long-term effects of a gas platform in the southern North Sea on the surrounding benthic community by both morphological and molecular identification of benthic species. The species composition and a set of abiotic factors of the sediment around a gas platform were assessed along four transects. Differences for the abiotic factors were found in the closer vicinity of the platform in the direction corresponding to the predominant currents. The number of benthic fauna families found in the molecular approach were on average three times higher than for the morphological approach. Both approaches showed that small differences occurred primarily due to changes in sedimentary organic matter content. Differences in species composition were more pronounced between transects rather than between distances from the platform.

Keywords: artificial structures, benthic ecology, decommissioning, epifauna, metabarcoding, North Sea

Introduction

Over the last decades man-made structures such as oil and gas platforms have become a widespread phenomenon in the North Sea. Through time, these structures often have become artificial reefs as they provide a solid substrate for sessile epifauna in areas

that are mainly characterized by soft sediment habitat (Whomersley and Picken, 2003; Krone *et al.*, 2013; Stap *et al.*, 2016). So far it is unknown whether artificial structures are beneficial for ecosystem functioning and biodiversity in a wider area surrounding these structures. This question is becoming

increasingly important as many platforms in the southern North Sea are about to be decommissioned in the coming decades. The contracted fate of offshore platforms at the end of their productive life is complete removal. However, arguments have been raised to leave parts or whole platforms in place as artificial reefs (Jørgensen, 2012) similarly as in the Gulf of Mexico and other parts of the United States (Fowler *et al.*, 2014; Claisse *et al.*, 2015).

The biomass of epifauna on submerged artificial structures may reach up to 500-fold the biomass found in the soft sediments (Picken *et al.*, 2000). Since the epifauna on new artificial substrates mainly consists of filter-feeders (e.g. mussels) this community may act as a biofilter, depleting primary organic matter in the water column while enriching it by producing faeces, nutrients, dissolved organics, and larvae (Krone *et al.*, 2013; Coates *et al.*, 2014). Consequently, water chemistry and particle composition are altered in the close vicinity (up to 100 m) of an artificial structure (Maar *et al.*, 2009). It is hence hypothesized that epifauna on oil and gas structures will cast a so-called “shadow”, over the soft sediment ecosystem near the structures, which influences food availability for surrounding benthic assemblages and impact species composition. Model calculations of the effect of mussels growing on a wind turbine (Maar *et al.*, 2009) demonstrated significant effects on the water column including depletion of water column chlorophyll in the wake of the structure which was subsequently confirmed by actual measurements. Beside these biogeochemical impacts, the physical presence of a structure will alter local hydrodynamics, which may influence sedimentological characteristics, changing erosion and deposition (Vanhellemont and Ruddick, 2014; Baeye and Fettweis, 2015; Carpenter *et al.*, 2016). Field studies on the impact of offshore gas platforms in the Adriatic Sea revealed a widespread effect (up to 1000 m) on the surrounding nematode community which was ascribed to physical alteration of the habitat (Fraschetti *et al.*, 2016), while the growth of a mussel population on the structure led in one case to the development of a mound of dead mussel shells close to the platform with consequences for the resident bottom fauna (Bomkamp *et al.*, 2004; Manoukian *et al.*, 2010).

A growing number of studies on the introduction of artificial hard substrate and their biological effects on the surrounding soft sediment environment is being published (Lindeboom *et al.*, 2011; Degraer and Brabant, 2012; Vandendriessche *et al.*, 2015; Coolen, 2017). So far, studies have mostly dealt with short-term effects (up to 5 years). Fouling communities on artificial structures, however, have been shown to change in composition when the structure remains intact long enough (Whomersley and Picken, 2003; Vandendriessche *et al.*, 2015). To aid the discussion of the effects of decommissioning of artificial structures, knowledge on the actual effects of a platform on its surroundings is necessary, for which a longer time-scale (multi-decadal) perspective is needed. Therefore, this study targets an >40-year-old offshore gas platform (L7A) in the southern North Sea and investigates whether this platform casts a “shadow” on the composition of the soft-sediment benthic communities. It was hypothesized that species composition in the wake of the platform, in the residual current direction, will differ from the species composition in reference areas. Besides species composition, a set of abiotic factors which are known to influence the benthic community composition were measured.

Most impact studies involving benthos by means of morphological identification only deal with macrofauna (Coates *et al.*,

2014; Coolen, 2017). Only when highly specialist knowledge is available, meiofauna is considered. Yet meiofauna organisms are, next to macrofauna, key indicators for ecosystem health (Balsamo *et al.*, 2012; Spilmont, 2013; Lallias *et al.*, 2014; Frascchetti *et al.*, 2016). This study applies both a classic morphological identification approach, via identification of macrofaunal specimens by a highly experienced taxonomist, and a molecular identification approach, via metabarcoding of DNA extracted from the entire benthic assemblage (including meiofauna and macrofauna), using a combination of next-generation sequencing and taxonomic affiliation based on reference libraries. The latter approach provides the opportunity to assess marine metazoan benthos in a new holistic and replicable manner (Chariton *et al.*, 2010; Taberlet *et al.*, 2012; Cowart *et al.*, 2015).

Methods

Study site and sampling design

The platform L7A in the southern North Sea was installed over 40 years ago. It was selected as study site, since non-toxic substances were used during past drilling operations and long-lasting effects due to toxic wastes can be excluded (Duineveld *et al.*, 2007). The platform is situated in a fauna-rich area with fine muddy sand (median grain size between 106 and 113 μm) at a water depth of 35 m (Duineveld *et al.*, 2007) (Figure 1). Based on the dominant residual current directions the shadow area was defined as the sector between 0° and 90° compass direction and the opposite direction between 180° and 270°.

Samples and measurements were collected during a cruise with RV Pelagia (7–12 May 2016). Sediment samples were collected at 150, 300, 450, and 600 m, respectively, from the platform along four transects in four perpendicular directions (SW, SE, NW, and NE) using the NIOZ box corer with a 706 cm² surface area and 55 cm sampling depth (equivalent to roughly 38 litres of sediment). From each box core one sub core (78 cm² surface) was retrieved for sedimentological analysis and stored at 4° C. Two surface samples for organic content measurements, taken of the surface of the box core with a spatula, were stored at –20° C. Samples for molecular identification (environmental DNA samples) were collected from the box core using a smaller core with a 5.60 cm² surface area and 10 cm sampling depth (equivalent to 56 ml of sediment) and immediately stored at –80° C. The remaining sediment in the box core was used for morphological identification and was sieved over a 1 mm round mesh sieve. Living organisms were sorted manually and identified up to the family level with a stereomicroscope by an experienced taxonomist (ML) according to Hartmann–Schröder (1996) and Hayward and Ryland (1995). Identified species were stored in 96% ethanol at ambient temperatures as reference samples for molecular methods.

Environmental variables

The sub cores taken for sedimentological analysis were opened in the laboratory and the top 10 cm was sliced into 1 cm slices. A subsample of each slice was cryodesiccated and subsequently left overnight in a water bath (100 ml) consisting of 0.1 M dispersing agent (Na₄P₂O₇) to prevent flocculation. Grain sizes were measured three times with a Beckman Coulter LS Particle Size Analyzer which measures a grain size distribution from 0.04 to 2000 μm for 117 size classes. Thereafter, measurements were divided into three size fractions: clay (<0.002 mm), silt

(0.002–0.05 mm), and sand (0.05–2 mm). Total organic carbon (TOC) was determined from the surface samples after removing inorganic carbonates by shaking samples twice in 2 M HCl (respectively for 4 and 12 h) followed by rinsing them twice with Milli-Q water. Subsequently, the sediments were cryodesiccated and thoroughly ground in an agate mortar. TOC and nitrogen (N) contents were measured on an Organic Elemental Analyser (Flash 2000, Thermo Scientific Bremen, Germany).

Reference library and mock sample

Genomic DNA from a subset of the morphological identified specimens (Supplementary Table S1) was extracted using the GenElute™ Mammalian Genomic DNA miniprep kit (Sigma-Aldrich Inc.) following the manufacturer's protocol. A 450 base pair (bp) part of nuclear small ribosomal subunit (18S) was amplified using the oligo-nucleotides F04 and R22 as primer pair (Sinniger *et al.*, 2016). The 18S gene was amplified in a 50 µl volume reaction, containing 0.6 µM of each primer, 0.2 µM dNTP, 800 ng/µl BSA, 1 U Phusion® High-Fidelity DNA Polymerase (Thermo Scientific Inc.), 1× Phusion® HF buffer (Thermo Scientific Inc.) and 5 µl of DNA extract. The thermal cycle conditions were as follows: an initial cycle of 30 s at 98°C; followed by 27 cycles, each comprised of 10 s at 98°C, 20 s at 60°C, and 30 s at 72°C, followed by a single cycle of 5 min at 72°C. The PCR products were Sanger sequenced in both directions on the ABI3730XL sequencer from Life Technologies by BaseClear (Leiden, Netherlands). Consensus sequences were complemented with their taxonomic data and stored as a local reference database.

One mock test sample was generated by combining DNA extractions from 21 species, representing 7 different phyla (Supplementary Table S1). The DNA extracts of the selected species were quantified on a Qubit 3.0 fluorimeter (Qiagen, Inc.) and were pooled in equimolar quantities. The mock sample served as a positive control throughout the 18S species identification process.

Molecular analysis

A subsample (10 g) was taken from each eDNA subcore at the following depth intervals: 0–2, 2–3, and 5–6 cm. DNA was extracted from these subsamples using the Powermax Soil™ DNA isolation kit (MoBio Inc.) following the manufacturer's instructions. DNA from all extractions, as well as a mock sample, were used as template to amplify, in triplicate, the 18S gene fragment as described in Methods section. All forward and reverse primers were extended with 12 nt unique barcodes. The PCR products were visually inspected after electrophoresis through a 1% agarose gel at 75 V for 50 min, excised from the gel, purified using the Qiaquick Gel Extraction Kit (Qiagen, Inc.) and quantified with a Qubit™ 3.0 fluorometer (Qiagen, Inc.). Samples were pooled in equimolar quantities together with blank PCR controls. The pooled sample was then subjected to a final purification using MinElute™ PCR Purification columns (Qiagen Inc.) as described by the manufacturer. The pooled sample was submitted for sequencing at Useq (Utrecht, Netherlands) on an Illumina MiSeq using the 2× 300 bp V3 kit.

Bioinformatics

Raw sequences were quality filtered using the *fastq_quality_filter* script in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Reads with a quality score ≤ 30 over 75% of the

positions were discarded. Quality filtered reads were de-multiplexed using the *split_libraries.py* script in QIIME (Caporaso *et al.*, 2010), allowing zero mismatches in both the forward and reverse primer. De-multiplexed sequences were then front, and end clipped to remove the primers. Reads were first de-replicated at a 100% similarity and sequences with less than 10 copies were discarded. The remaining unique sequences were clustered using a 95% similarity cut-off in VSEARCH (Rognes *et al.*, 2016). Taxonomic assignments were performed against the SILVA 18S rRNA database (release 119, www.arb-silva.de; Pruesse *et al.*, 2007) using the RDP Classifier (Wang *et al.*, 2007) with a minimum confidence of 0.5. At a confidence level of 0.5 all OTUs found for the mock sample could be assigned at the family level to one of the species added to this sample.

Data analysis

For the morphological approach, count data were merged into taxonomic families when possible, resulting in a total number of individuals per taxonomic family. As the number of reads for the molecular approach have a weak relationship with biomass or abundance (Elbrecht and Leese, 2015), all OTU data were converted to presence or absence prior to further analysis (Chariton *et al.*, 2015). OTUs were first merged per location (i.e. depth-subsamples were averaged at each location) and subsequently combined into taxonomic families. The correlation between the number of metazoan OTUs and read depth was tested to avoid a bias introduced by sampling effort and no correlations were found (Pearson, $r = -0.176$, $p = 0.515$). Also, rarefaction curves (using R package *vegan/rarecurve*, $step = 20$) showed a saturation for all samples (Supplementary Figure S2). Therefore, no transformation of OTU abundances was necessary and the number of OTUs per taxonomic families were used throughout subsequent analyses. Shannon–Wiener diversity estimates were calculated using the count data for the morphological approach and the number of OTUs per taxonomic family for the molecular approach.

Since it was hypothesized that the platform could exert an effect on the benthic community via a change in sediment composition, the metazoan data were screened for potential indicators of a change in grain size, TOC, and N. Taxa were designated as potential indicator taxa if the number of individuals (morphological approach) or the number of OTUs (molecular approach) within a taxonomic family were correlated to either the percentage of silt, TOC, or N. A taxonomic family was designated as potential indicator taxa if the correlation coefficient rho was found to be higher than 0.4 or lower than -0.4 and at least 10 species or OTUs were found within this family for all samples combined.

The number of individuals for the morphological approach and the number of OTUs for the molecular approach within a taxonomic family were subjected to Hellinger transformation (Legendre and Gallagher, 2001) using the *vegan* function “*decostand*”. A screeplot was made to check for variances of the ordination components and to determine the optimal number of dimensions or axis in the multidimensional scaling. Non-metric multidimensional scaling using Bray–Curtis dissimilarity distances at two dimensions for the morphological approach and three dimensions for the molecular approach were performed. The Bray–Curtis dissimilarity matrix was further used for analysis of variance between the transects (permanova) using the *adonis2* function and for the simpler analysis to discriminate the effect of each species. All data were analysed in R v3.4.3.

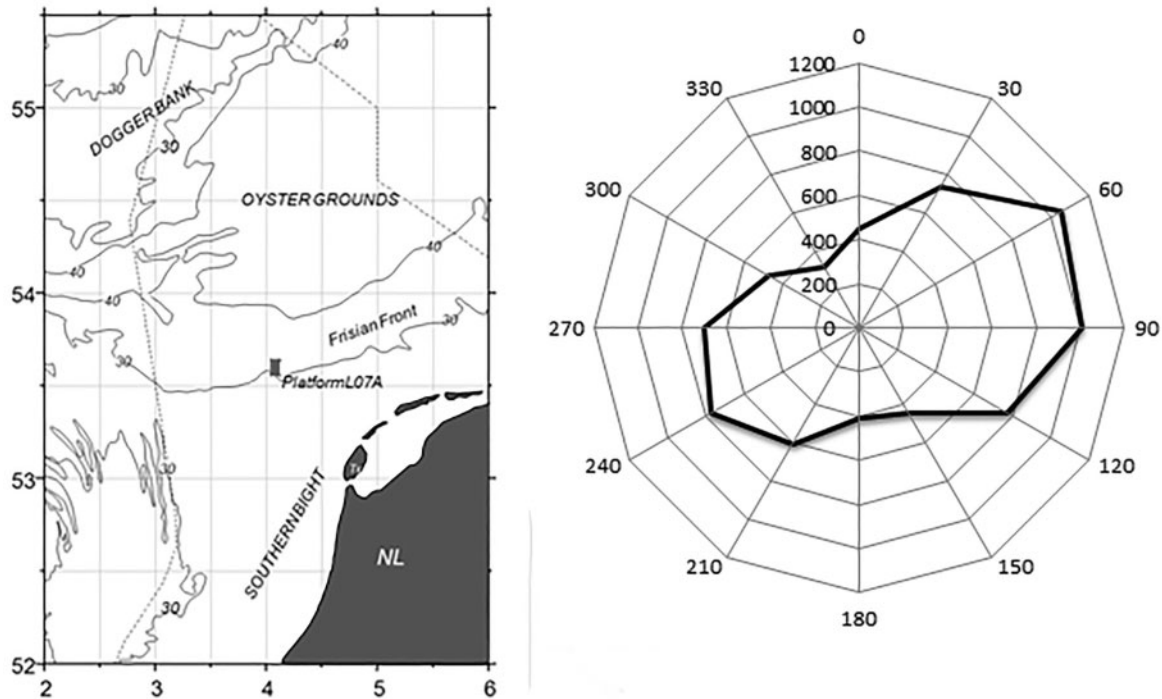


Figure 1. Sample locations were based around the L-7A gas-platform in the southern North Sea (left panel). Sample locations were distributed along four different transects; north-east (45°), south-east (135°), south-west (225°) and north-west (315°). Along each transect a box core was taken at 150, 300, 450 and 600 m from the platform. The right panel shows the sum of current vectors in the different directions as measured during the scientific cruise in 2017. The dominant current direction was between $0-90^\circ$ (flood) and $180-270^\circ$ (ebb), which were designated as shadow areas. Areas between $90-180$ and $270-360^\circ$ were designated as reference areas.

Results

Validation of taxonomic assignment

After sequence quality control, a total of 85 923 reads were assigned to the mock sample. These reads resulted in 490 OTUs after clustering at a threshold at $>95\%$. Only 1 out of the 21 species added to the mock sample was not recovered, i.e. *Ampelisca brevicornis*. False positives were found below a relative read abundance of 0.001%. This relative read abundance was then used as cut-off in the further data analysis of the 16 environmental samples.

Taxonomic composition

Classic morphological analysis of the box core samples, sieved over a 1-mm sieve and identified by stereo microscope, yielded a total of 1264 specimens belonging to 43 taxonomic families for the 16 samples. In total, seven metazoan phyla were found, of which only four were found at all locations. Most individuals belonged to the echinoderms (52%), and annelids (20%) (Figure 2, upper panel). The most abundant families were the echinoderm family Amphiuridae, the annelid family Lumbrineridae, the phoronid family Phoronidae, and the arthropod family Callianassidae.

The Illumina sequencing of the molecular samples generated 6.4 million reads after quality filtering for the 16 samples, of which 22% could be confidently assigned to metazoans. Clustering resulted in 544 metazoan operational taxonomic units (OTUs), the number of OTUs ranged from 121 to 323 for individual samples. The molecular approach recovered many more metazoan phyla than the morphological approach; 16 in the combined samples. Ten of these phyla were recovered from each

location (Figure 2, lower panel). The metazoan diversity derived from the molecular approach was largely dominated by nematodes, which formed the most diverse group (11% of all OTUs). The next most diverse phyla were the arthropods (5%), and annelids (2%). Of all metazoan OTUs, 60% could be taxonomically classified at the family level. The most abundant metazoan families based on the number of OTUs were the nematode families Comesomatidae, Oxystominidae, and Loxoconchidae, followed by the arthropod family Temoridae and the annelid family Lumbrineridae.

Biodiversity and environmental variables

Percentage clay was very low (between 0% and 0.2%) for all locations, therefore, instead percentage silt was used as a measure for further grain size analyses. Percentage silt was highest in the NE transect with a mean percentage of $30.43 \pm 1.9\%$ (Figure 3; Supplementary Table S3) and lowest in the SE transect ($24.88 \pm 4.78\%$). The percentage silt tended to increase with increasing distance from the platform. However, only for the SW transect this trend was significant (Pearson, $r = 0.965$, $p = 0.035$). The levels of TOC and N were on average highest in the NE transect (TOC: $0.566 \pm 0.19\%$; N: $0.047 \pm 0.01\%$) (Figure 3; Supplementary Table S3). Similarly as the percentage of silt, TOC and N levels showed an increase with increasing distance from the platform, however, correlations were not statistically significant.

The Shannon–Wiener index based on the numbers of specimen per family found from the morphological identification approach was negatively correlated with the percentages of TOC, N,

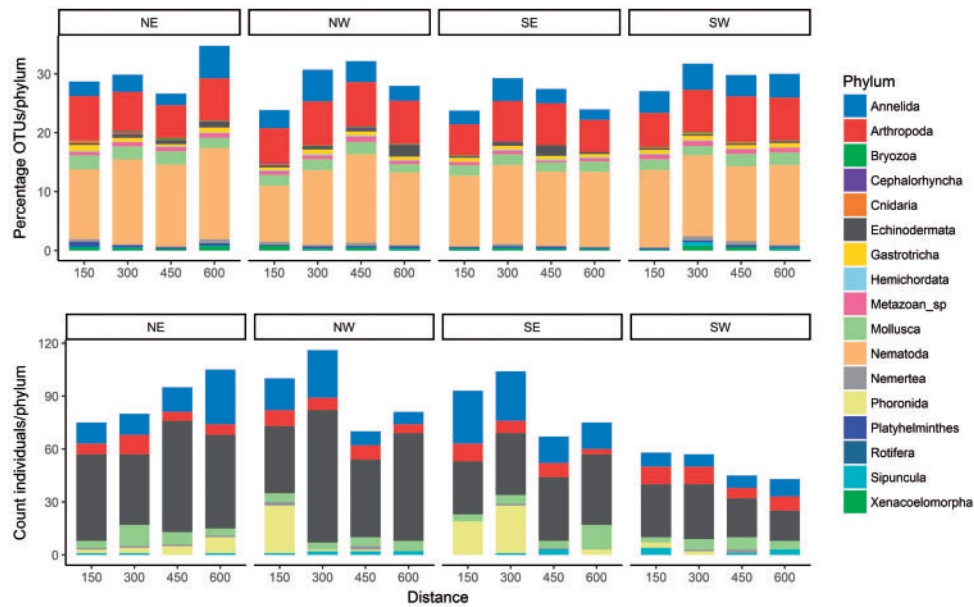


Figure 2. Taxonomic composition per location (Distance in m from the platform). For each of the samples grouped per transect the proportions of phyla identified through the morphological approach (upper panel) or the molecular approach (lower panel). The number of individuals per phylum was used for the morphological approach and number of OTUs within a phylum was calculated for the molecular approach.

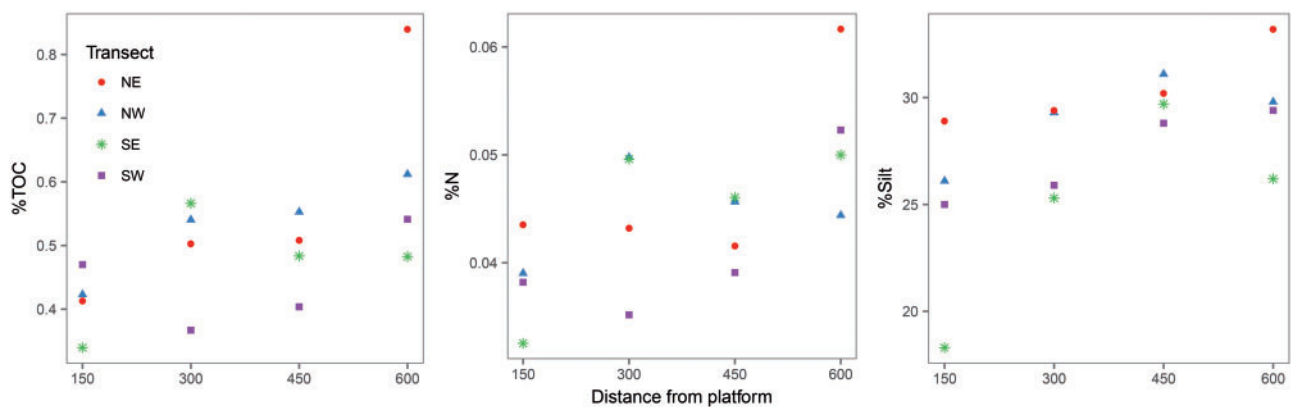


Figure 3. Environmental variables for the various sampling locations. The percentage of carbon and nitrogen from surface sediments and the average percentage of silt from the top 10 cm of sediment is shown for each sample location.

and silt (Figure 4, upper panel). However, only the relationship with the percentage silt was statistically significant (Pearson, $r = -0.626$, $p = 0.010$). The Shannon–Wiener index based on the number of OTUs per family found in the molecular approach was twice as high as the index derived from the morphological approach. The correlations between Shannon–Wiener index values from the molecular approach and the environmental variables were less strong than the correlations with index values based on the morphological approach and none of these were significant (Figure 4, lower panel). The Shannon–Wiener index based on the molecular approach increased slightly with increasing percentages of silt as opposed to the decreasing trend found with values based on the morphological approach.

Potential indicator taxa

Within the dataset based on the morphological approach, the arthropod family Upogebiidae was significantly negatively

correlated with the percentage of N (Pearson $r = -0.525$, $p = 0.037$) and was therefore designated as potential indicator taxa (Table 1). The echinoderm family Amphiuiridae, the annelid family Lumbrineridae, and the Phoronidae were found in high abundances with still a moderate correlation with one or multiple of the environmental variables ($r > 0.4$). The Amphiuiridae and Lumbrineridae were positively affected by higher organic content, either TOC or N. Whereas the Amphiuiridae were found in higher abundances with higher percentages of silt, the Phoronidae were found in lower abundances in relation to higher silt contents.

The Amphiuiridae family was the only family designated as indicator taxon by both the morphological and molecular approach. The abundance of Amphiuiridae in the molecular approach was positively correlated with the percentage of TOC. The molecular approach also identified several meiofauna families as potential indicator taxa. The number of OTUs within the nematode families Thoracostomopsidae and Cyatholaimidae

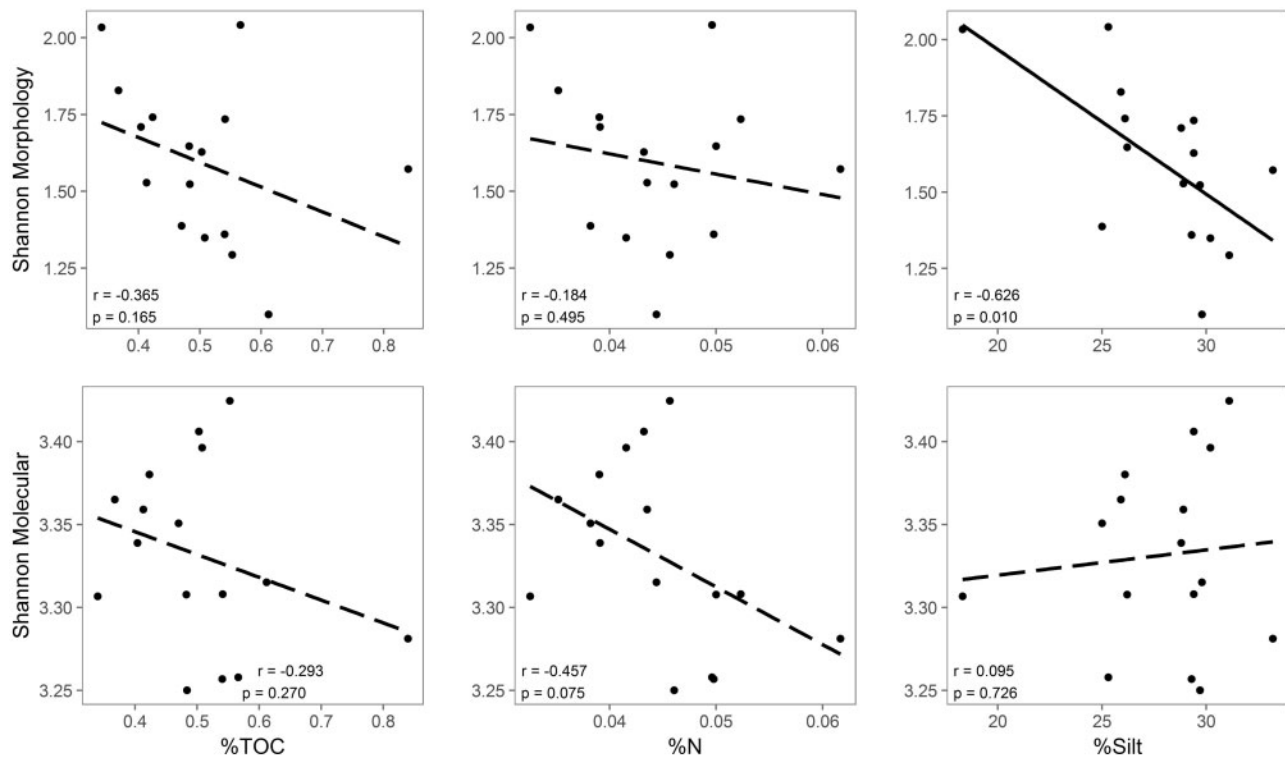


Figure 4. Relationships between the biodiversity and environmental variables. The Shannon–Wiener index was calculated from number of individuals within a taxonomic family for the morphological approach (top panel) or the number of OTUs within a taxonomic family for the molecular approach (bottom panel) and was plotted against the percentage of carbon, nitrogen and silt found at the sample location.

Table 1. Potential indicator taxa.

Approach	Family	N	TOC		N		Silt	
			Rho	P	Rho	P	Rho	P
Morpho	Upogebiidae (Ar)	23	-0.489	0.055	-0.525	0.037*		
	Nuculidae (M)	26	-0.479	0.061	-0.477	0.062		
	Amphiuridae (E)	641	0.458	0.074			0.449	0.093
	Lumbrineridae (An)	116			0.411	0.113		
	Phoronida (P)	102					-0.405	0.134
Molecular	Camacoloimidae (N)	14	-0.644	0.007*	-0.495	0.051	-0.483	0.058
	Thoracostomopsidae (N)	108	0.538	0.031*	–	–	–	–
	Cyantholaimidae (N)	179	0.538	0.032*	–	–	–	–
	Scalibregmatidae (An)	25	–	–	–	–	-0.532	0.034*
	Loxoconchidae (Ar)	211	0.519	0.039*	0.546	0.029*	0.459	0.074
	Corbulidae (M)	33	–	–	0.510	0.044*	–	–
	Linhomoeidae (N)	64	–	–	–	–	-0.504	0.046*
	Amphiuridae (E)	89	0.496	0.051	–	–	–	–
	Miraciidae (Ar)	17	–	–	–	–	0.466	0.069
	Semelidae (M)	25	–	–	-0.420	0.105	–	–
	Calanidae (Ar)	12	-0.411	0.114	-0.418	0.107	–	–
Xyalidae (N)	135	–	–	-0.417	0.108	–	–	

Correlation tests were performed for the abundance of specimens within a taxonomic family for the morphological approach and the number of OTUs within a taxonomic family for the molecular approach against the environmental variables. Only families showing a strong or moderate correlation ($r > 4$) and an abundance (N) above 10 are presented here. An = Annelida, Ar = Arthropoda, E = Echinodermata, M = Mollusca, N = Nematoda, P = Phoronida.

showed a positive correlation with TOC levels (Pearson, $r = 0.538$, $p = 0.031$ and $r = 0.538$, $p = 0.032$, respectively), whereas nematodes from the family Camacoloimidae were negatively correlated with TOC levels (Pearson, $r = -0.644$,

$p = 0.007$). For the other phyla, the arthropod family Loxoconchidae was positively correlated to both TOC and N levels (Pearson, $r = 0.519$, $p = 0.039$ and $r = 0.546$, $p = 0.029$) and the mollusc family Corbulidae was positively correlated to the

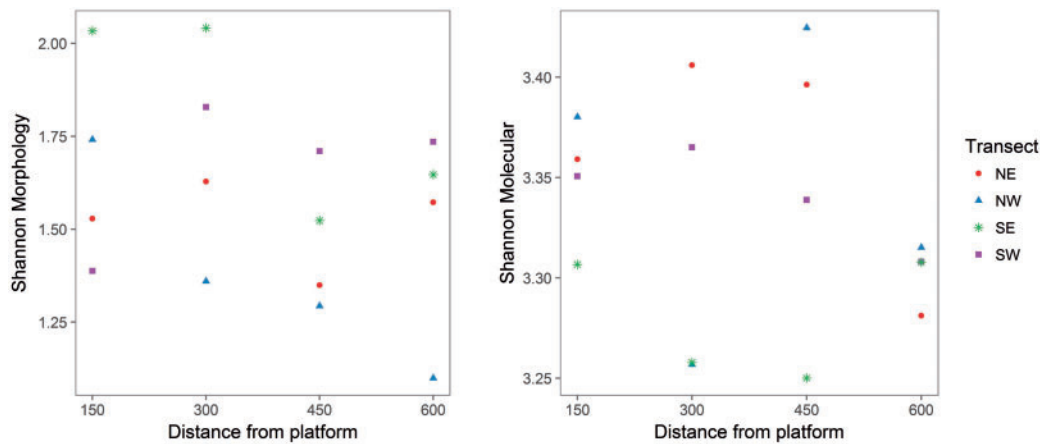


Figure 5. Biodiversity per sample location. The Shannon–Wiener index was calculated from the number of individuals within a taxonomic family for the morphological approach (left panel) or the number of OTUs within a taxonomic family for the molecular approach (right panel) and was plotted for each sample location per transect.

levels of nitrogen (Pearson, $r = 0.510$, $p = 0.044$). The annelid family Scalibregmatidae and the nematod family Linhomoeidae were negatively correlated to the percentage silt (Pearson, $r = -0.532$, $p = 0.034$ and $r = -0.504$, $p = 0.046$, respectively).

Comparison between sample locations

The values of the Shannon–Wiener index varied between sample locations for both the morphological and molecular approach (Figure 5; Supplementary Table S4). The Shannon values based on the morphological approach showed the strongest trend along the NW transect, for which diversity decreased with increasing distance from the platform (Pearson, $r = -0.956$, $p = 0.044$). In contrast, diversity was stable with increasing distances on the NE transect (Pearson, $r = -0.156$, $p = 0.844$) and diversity in this transect showed the lowest variation along the distances ($\mu = 1.520 \pm 0.121$). The Shannon values based on the molecular approach showed a negative correlation with distance on all transects, however, none of these were statistically significant. The MDS ordination for the morphological approach showed a deviating composition of macrofauna on the SW transect with all its data points separated from the remaining samples (Figure 6, left panel). A permanova analysis indicated a statistically significant difference between the transects ($F_{3,12} = 1.547$, $p = 0.026$). Simper analysis showed that this deviation was mainly due to a lower abundance of the echinoderm family Amphiuridae and the phylum Phoronida on the SW transect. An MDS ordination for the molecular approach combined with a permanova analysis resulted in a significant difference between the benthic communities for the different transects ($F_{3,12} = 1.497$, $p = 0.040$) but not between the distances from the platform ($F_{3,12} = 1.064$, $p = 0.366$) (Figure 6, right panel).

Discussion

The aim of the present study was to seek evidence for an effect of an offshore gas platform on the composition of the surrounding metazoan communities. The supposed effect of the platform was hypothesized to be primarily due to the long established epifaunal community which acts as a biofilter and casts a shadow over its surroundings (Van der Stap *et al.*, 2016). The methodology that

was applied consisted of a classical morphological approach targeting only macrofauna and a molecular approach which also include the smaller meiofauna.

Prominent variation was found in the grain size characteristics in the environment surrounding the gas platform. A higher percentage of silt was found on the transect in the residual current direction, i.e. in the “shadow” area of the structure, while coarser sediment was observed in the close vicinity of the artificial structure. Similar grain size effects have been found around other artificial structures and were interpreted to reflect changes in velocity and direction of water movement (Mendoza and Henkel, 2017). The strongest correlation between distance from the platform and grain sizes were found in the SW and NE transects, aligning with the directions of the dominant residual currents. TOC and nitrogen (N) levels showed equivocal trends around the platform. The organic content of the surface sediment was on average highest in the NE transect, which is in line with the distribution of silt. The combined data suggests a redistribution of silt and associated organic matter in the direction of the residual current from SW to NE (Heery *et al.*, 2017). For the low percentages of TOC and N in the vicinity of the platform two explanations are proposed: the first is in line with the original hypothesis of this project, i.e. depletion of the organic content of water due to the biofilter effect of the epifauna (Davis *et al.*, 1982; Maar *et al.*, 2009). The other (mechanistic) explanation evokes scouring of the sediment caused by the acceleration of the flow by the platform structure and deposition of fine particles further from the platform (Rudolph *et al.*, 2004).

The Shannon–Wiener index is a commonly used index for comparing benthic communities analysed by classical morphology approaches (Gray, 2000; Patrício *et al.*, 2009). More recently it has also been used in connection with molecular approaches (Lanzén *et al.*, 2016). However, the outcomes of these indices are not necessarily comparable. First, there is a difference between the sampling procedure in the two approaches in terms of sampling volume and size fraction of the fauna. In the morphological approach, a fixed surface area is sampled and a cut-off size selection is applied by sieving. Moreover, only organisms of this size class that are present at the time of sampling will be collected. Environmental DNA, on the contrary, can persist in the

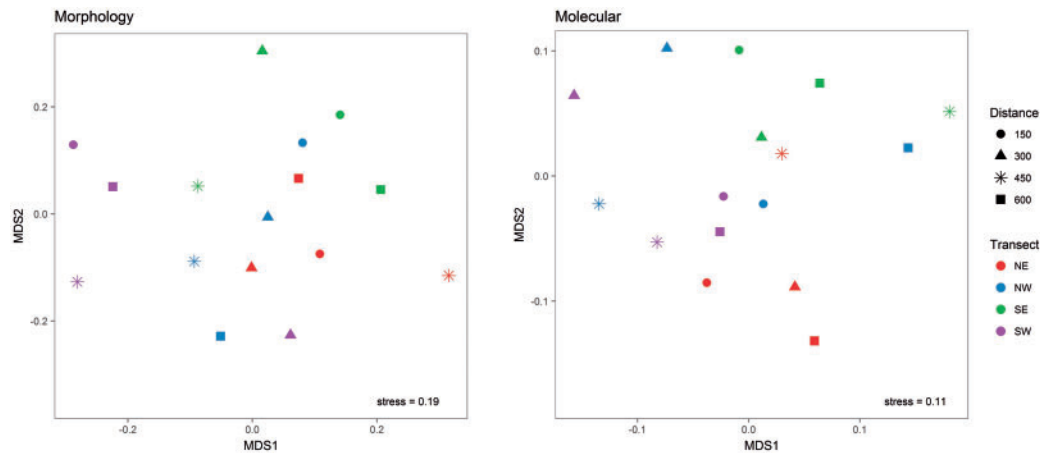


Figure 6. Nonmetric multidimensional scaling (nMDS) plot for community composition. The nMDS is based on Bray–Curtis dissimilarities of community composition. Composition was based on the number of individuals per taxonomic family for the morphological approach (left panel) and the number OTUs per taxonomic family for the molecular approach (right panel).

environment over time and therefore will reflect present and past presences of fauna, and possibly even presences from organisms in the wider area (Dell’Anno *et al.*, 2002). In addition, the molecular approach includes fauna from all size classes, which likely increases taxonomic richness.

Additionally, abundance estimates used for the calculation of the Shannon–Wiener index are different in the morphological and molecular approach and hence also the meaning of the index differs. Abundance estimates in the morphological approach are counts of specimens belonging to a specific taxon, while the molecular approach in this study uses OTU abundance within a taxon (here family). OTU abundance has been shown to increase with increasing numbers of specimens analysed within a taxonomic group due to polymorphism (Dell’Anno *et al.*, 2015). However, this genetic diversity is not necessarily similar for all taxa. Whilst the Shannon–Wiener index is sensitive to the number of taxa (richness) in both approaches, it expresses evenness in the distribution of specimens across taxa in the classical approach as opposed to differential genetic diversity within taxa in the molecular approach. Despite the different meaning of the Shannon–Wiener index, Lejzerowicz *et al.* (2015) already showed the applicability of biotic indices, and in particular the Shannon–Wiener index for the molecular approach. An extensive review of the use of biotic indices for molecular approaches has been performed by Pawlowski *et al.* (2018). Here, we only touched upon the essentials relevant to this study.

Potential indicator taxa for the effects of changing organic content or silt levels could be indicated. For both the molecular and morphological approach more taxa correlated positively than negatively to the organic content. For both approaches, the abundance of the macrofaunal family Amphiruridae increased with increasing organic content levels. Species in the Amphiruridae family are rapid growing with a high metabolic rate and hence have high food requirements and thrive under high food conditions (Buchanan, 1964; Josefson and Jensen, 1992). In this study, lowest abundances for the Amphiruridae were found in the SW transect and highest in the NE transect, which is in line with the distribution of organic carbon and silt.

Although macrofauna species have been used as key indicators for environmental health for decades, recent developments in

terms of molecular techniques have made it easier to also assess the function of meiofauna as bio-indicators (Fonseca *et al.*, 2014; Lallias *et al.*, 2014; Chariton *et al.*, 2015; Lanzén *et al.*, 2016). Meiofauna species are considered to be suitable indicators for marine ecosystem monitoring due to their relatively high abundance and their complex interplay with other faunal species (Sutherland *et al.*, 2007; Balsamo *et al.*, 2012). Abundances of certain nematode species have been shown to increase at slightly elevated levels of organic content (Gee *et al.*, 1985) and also in this study, two nematode families showed a positive correlation with TOC and N levels. Relations between arthropods and environmental variables in the study were less clear. One meiofaunal arthropod family of ostracods, the Loxoconchidae, showed a positive relationship between OTU numbers and increasing TOC and N levels, whereas the copepod family Calanidae showed a negative relationship with organic content levels.

Previous studies have investigated the influences of man-made structures on the surrounding benthic environment by either a morphological identification approach (Danovaro *et al.*, 2002; Terlizzi *et al.*, 2008; Manoukian *et al.*, 2010; Coates *et al.*, 2014; Frascetti *et al.*, 2016) or a molecular identification approach (Lanzén *et al.*, 2016). This study is the first to date to encompass both approaches. An obvious advantage of the morphological approach is the ability to provide actual species abundance data, whereas metabarcoding datasets are still limited to presence/absence data of OTUs (Deagle *et al.*, 2013; Cowart *et al.*, 2015; Ficetola *et al.*, 2015; Piñol *et al.*, 2015). A good example is the phylum Phoronida. This phylum consists of only one family Phoronidae (WoRMS, 2018) with few species and thus molecular diversity within this phylum is nihil. Even though the morphological approach found high numbers of Phoronidae, in this study, the abundance of different OTUs within the Phoronidae in the molecular approach was low. On the other hand, the most noticeable disadvantage of the morphological approach is the taxonomic expertise needed for fauna identification. Because of this, meiofauna species are often excluded from environmental impact studies. Since molecular methods, such as metabarcoding, include macrofauna and meiofauna, they provide a more holistic view of the benthic community composition (Taberlet *et al.*, 2012; Chariton *et al.*, 2015; Lanzén *et al.*, 2016; Sinniger *et al.*, 2016). In

this study, the number of families found in the molecular approach were on average three times higher compared with the morphological approach. The additional families found were mainly meiofauna families, taxa which are potential bio-indicators of changing environmental conditions.

The analysis of indicator taxa and the analysis of biotic indices from both the morphological and molecular approach showed that differences in abundance within taxonomic families can occur due to abiotic changes. As the particular platform has been established several decades ago, species composition on the platform was supposed to represent a mature community and therefore is a good measure of the long-term effects of artificial structures on their immediate surroundings. Differences within the surrounding communities and abiotic factors were most noticeable between the NE and SW transect, which are the downstream and upstream directions of the strongest currents around the platform, respectively. Differences in community composition were most pronounced between the transects rather than within a transect at varying distances from the platform. This would suggest that the presence of the platform has evoked changes in soft bottom communities. However, it was not possible to disentangle the biological effects of epifauna from the physical effect of the platform itself. Likely both factors are involved. Either way, the complete removal of the platform as part of the decommissioning process will alter the current benthic species composition.

Supplementary data

[Supplementary material](#) is available at the *ICESJMS* online version of the manuscript.

Acknowledgements

We are grateful to captain, crew and technicians for their help on board RV Pelagia. We thank the INSITE programme for funding the scientific SHADOW project. Royal NIOZ and INSITE have funded the scientific cruise. We thank Total for granting permission to work around platform L7A. The morphological identification on board greatly benefited from the efforts of Ulrike Hanz, whereas the work in the molecular laboratory could not have succeeded without the help of Harry Witte. A. Filippidi was financially supported by the INSITE programme.

References

- Baeye, M., and Fettweis, M. 2015. In situ observations of suspended particulate matter plumes at an offshore wind farm, southern North Sea. *Geo-Marine Letters*, 35: 247–255.
- Balsamo, M., Semprucci, F., Frontalini, F., and Coccioni, R. 2012. Meiofauna as a tool for marine ecosystem biomonitoring. *Marine Ecosystems*, 310: 77–104.
- Bomkamp, R. E., Page, H. M., and Dugan, J. E. 2004. Role of food subsidies and habitat structure in influencing benthic communities of shell mounds at sites of existing and former offshore oil platforms. *Marine Biology*, 146: 201–211.
- Buchanan, J. B. 1964. A comparative study of some features of the biology of *Amphiura filiformis* and *Amphiura chiajei* [Ophiuroidea] considered in relation to their distribution. *Journal of the Marine Biological Association of the United Kingdom*, 44: 565.
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., and Fierer, N. and 2010. QIIME allows high throughput community sequencing data. *Nature Methods*, 7: 335–336.
- Carpenter, J. R., Merckelbach, L., Callies, U., Clark, S., Gaslikova, L., and Baschek, B. 2016. Potential impacts of offshore wind farms on North Sea stratification. *PLoS One*, 11: e0160830–e0160828.
- Chariton, A. A., Court, L. N., Hartley, D. M., Colloff, M. J., and Hardy, C. M. 2010. Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. *Frontiers in Ecology and the Environment*, 8: 233–238.
- Chariton, A. A., Stephenson, S., Morgan, M. J., Steven, A. D. L., Colloff, M. J., Court, L. N., and Hardy, C. M. 2015. Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. *Environmental Pollution*, 203: 165–174.
- Claisse, J. T., Pondella, D. J., Love, M., Zahn, L. A., Williams, C. M., and Bull, A. S. 2015. Impacts from partial removal of decommissioned oil and gas platforms on fish biomass and production on the remaining platform structure and surrounding Shell mounds. *PLoS One*, 10: e0135812–e0135819.
- Coates, D. A., Deschutter, Y., Vincx, M., and Vanaverbeke, J. 2014. Enrichment and shifts in macrobenthic assemblages in an offshore wind farm area in the Belgian part of the North Sea. *Marine Environmental Research*, 95: 1–12.
- Coolen, J. 2017. North Sea reefs: benthic biodiversity of artificial and rocky reefs in the southern North Sea, Doctoral dissertation, chapter 4.
- Cowart, D. A., Pinheiro, M., Mouchel, O., Maguer, M., Grall, J., Miné, J., and Arnaud-Haond, S. 2015. Metabarcoding is powerful yet still blind: a comparative analysis of morphological and molecular surveys of seagrass communities. *PLoS One*, 10: e0117562–e0117526.
- Danovaro, R., Gambi, C., Mazzola, A., and Mirto, S. 2002. Influence of artificial reefs on the surrounding infauna: analysis of meiofauna. *ICES Journal of Marine Science*, 59: S356–S362.
- Davis, N., VanBlaricom, G. R., and Dayton, P. K. 1982. Man-made structures on marine sediments: effects on adjacent benthic communities. *Marine Biology*, 70: 295–303.
- Deagle, B. E., Thomas, A. C., Shaffer, A. K., Trites, A. W., and Jarman, S. N. 2013. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? *Molecular Ecology Resources*, 13: 620–633.
- Degraer, S., Brabant, R., and Rumes, B. (eds). 2012. Offshore wind farms in the Belgian part of the North Sea: Heading for an understanding of environmental impacts. Royal Belgian Institute of Natural Sciences, Management Unit of the North Sea Mathematical Models, Marine Ecosystem Management Unit: Brussel. 155 pp.
- Dell'Anno, A., Bompadre, S., and Danovaro, R. 2002. Quantification, base composition, and fate of extracellular DNA in marine sediments. *Limnology and Oceanography*, 47: 899–905.
- Dell'Anno, A., Carugati, L., Corinaldesi, C., Riccioni, G., and Danovaro, R. 2015. Unveiling the biodiversity of deep-sea nematodes through metabarcoding: are we ready to bypass the classical taxonomy? *PLoS One*, 10: e0144928–e0144918.
- Duineveld, G. C. A., Bergman, M. J. N., and Lavaleye, M. S. S. 2007. Effects of an area closed to fisheries on the composition of the benthic fauna in the southern North Sea. *ICES Journal of Marine Science*, 64: 899–810.
- Elbrecht, V., and Leese, F. 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass-sequence relationships with an innovative metabarcoding protocol. *PLoS One*, 10: e0130324–e0130316.
- Ficetola, G. F., Pansu, J., Bonin, A., Coissac, E., Giguët-Covex, C., De Barba, M., Gielly, L., et al. 2015. Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources*, 15: 543–556.
- Fonseca, V. G., Carvalho, G. R., Nichols, B., Quince, C., Johnson, H. F., Neill, S. P., Lambshead, J. D., et al. 2014. Metagenetic analysis

- of patterns of distribution and diversity of marine meiobenthic eukaryotes. *Global Ecology and Biogeography*, 23: 1293–1302.
- Fowler, A. M., Macreadie, P. I., Jones, D. O. B., and Booth, D. J. 2014. Ocean & coastal management: A multi-criteria decision approach to decommissioning of offshore oil and gas infrastructure. *Ocean and Coastal Management*, 87: 20–29.
- Fraschetti, S., Guarnieri, G., Gambi, C., Bevilacqua, S., Terlizzi, A., and Danovaro, R. 2016. Impact of offshore gas platforms on the structural and functional biodiversity of nematodes. *Marine Environmental Research*, 115: 56–64.
- Gee, J. M., Warwick, R. M., Schaanning, M., Berge, J. A., and Ambrose, W. G. 1985. Effects of organic enrichment on meiofaunal abundance and community structure in sublittoral soft sediments. *Journal of Experimental Marine Biology and Ecology*, 91: 247–262.
- Gray, J. 2000. The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and Ecology*, 250: 23–49.
- Hartmann-Schröder, G. 1996. Annelida, Borstenwürmer, Polychaeta. 2nd revised ed. *Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise*, 58. Gustav Fischer, Jena. 648 pp.
- Hayward, P. J., and Ryland, J. S. (eds). 1995. *Handbook of the Marine Fauna of North-West Europe*. Oxford University/Oxford University Press, Oxford, 800 pp.
- Heery, E. C., Bishop, M. J., Critchley, L. P., Bugnot, A. B., Airoldi, L., Mayer-Pinto, M., Sheehan, E. V., et al. 2017. Identifying the consequences of ocean sprawl for sedimentary habitats. *Journal of Experimental Marine Biology and Ecology*, 492: 31–48.
- Jørgensen, D. 2012. OSPAR's exclusion of rigs-to-reefs in the North Sea. *Ocean and Coastal Management*, 58: 57–61.
- Josefson, A. B., and Jensen, J. N. 1992. Growth patterns of *Amphiura filiformis* support the hypothesis of organic enrichment in the Skagerrak-Kattegat area. *Marine Biology*, 112: 615–624.
- Krone, R., Gutow, L., Joschko, T. J., and Schröder, A. 2013. Epifauna dynamics at an offshore foundation e Implications of future wind power farming in the North Sea. *Marine Environmental Research*, 85: 1–12.
- Lallias, D., Hiddink, J. G., Fonseca, V. G., Gaspar, J. M., Sung, W., Neill, S. P., Barnes, N., et al. 2014. Environmental metabarcoding reveals heterogeneous drivers of microbial eukaryote diversity in contrasting estuarine ecosystems. *The ISME Journal*, 9: 1208–1221.
- Lanzén, A., Lekang, K., Jonassen, I., Thompson, E. M., and Troedsson, C. 2016. High-throughput metabarcoding of eukaryotic diversity for environmental monitoring of offshore oil-drilling activities. *Molecular Ecology*, 25: 4392–4406.
- Legendre, P., and Gallagher, E. D. 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia*, 129: 271–280.
- Lejzerowicz, F., Esling, P., Pillet, L., Wilding, T. A., Black, K. D., and Pawlowski, J. 2015. High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems. *Scientific Reports*, 5: 13932.
- Lindeboom, H. J., Kouwenhoven, H. J., Bergman, M. J. N., Bouma, S., Brouwer, S., Daan, R., Fijn, R. C., et al. 2011. Short-term ecological effects of an offshore wind farm in the Dutch coastal zone; a compilation. *Environmental Research Letters*, 6: 035101.
- Maar, M., Bolding, K., Petersen, J. K., Hansen, J. L. S., and Timmermann, K. 2009. Local effects of blue mussels around turbine foundations in an ecosystem model of Nysted off-shore wind farm, Denmark. *Journal of Sea Research*, 62: 159–174.
- Manoukian, S., Spagnolo, A., Scarcella, G., Punzo, E., Angelini, R., and Fabi, G. 2010. Effects of two offshore gas platforms on soft-bottom benthic communities (northwestern Adriatic Sea, Italy). *Marine Environmental Research*, 70: 402–410.
- Mendoza, M., and Henkel, S. K. 2017. Benthic effects of artificial structures deployed in a tidal estuary. *Plankton and Benthos Research*, 12: 179–189.
- Patrício, J., Neto, J. M., Teixeira, H., Salas, F., and Marques, J. C. 2009. The robustness of ecological indicators to detect long-term changes in the macrobenthos of estuarine systems. *Marine Environmental Research*, 68: 25–36.
- Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéoz-Perret-Gentil, L., Beja, P., Boggero, A., Borja, A., et al. 2018. The future of biotic indices in the ecogenomic era: integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. *Science of the Total Environment*, 637–638: 1295–1310.
- Picken, G., Baine, M., Heaps, L., and Side, J. 2000. Rigs to reefs in the North Sea. In *Artificial Reefs in European Seas*. Springer Netherlands, Dordrecht, pp. 331–342.
- Piñol, J., Mir, G., Gomez-Polo, P., and Agustí, N. 2015. Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. *Molecular Ecology Resources*, 15: 819–830.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., and Glöckner, F. O. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, 35: 7188–7196.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4: e2584.
- Rudolph, D., Bos, K., Lujendijk, A., Rietema, K., and Out, J. 2004. Scour Around Offshore Structures, Analysis of Field Measurements. In: Chiew, Y. M., Lim, S. Y., Cheng, N. S. (eds) *Proceedings 2nd International Conference Scour and Erosion, Nanyang Technology Univ.*, Maritime Research Centre, Singapore, pp. 400–407.
- Sinniger, F., Pawlowski, J., Harii, S., Gooday, A. J., Yamamoto, H., Chevallon, P., Cedhagen, T., et al. 2016. Worldwide analysis of sedimentary DNA reveals major gaps in taxonomic knowledge of deep-sea benthos. *Frontiers in Marine Science*, 3: 1–14.
- Spilmont, N. 2013. The future of benthic indicators: moving up to the intertidal. *Open Journal of Marine Science*, 03: 76–86.
- Van Der Stap, T., Coolen, J. W. P., and Lindeboom, H. J. 2016. Marine fouling assemblages on offshore gas platforms in the southern North Sea: effects of depth and distance from shore on biodiversity. *PLoS One*, 11: e0146324.
- Sutherland, T. F., Levings, C. D., Petersen, S. A., Poon, P., and Piercey, B. 2007. The use of meiofauna as an indicator of benthic organic enrichment associated with salmonid aquaculture. *Marine Pollution Bulletin*, 54: 1249–1261.
- Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., and Willerslev, E. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, 21: 2045–2050.
- Terlizzi, A., Bevilacqua, S., Scuderi, D., Fiorentino, D., Guarnieri, G., Giangrande, A., Licciano, M., et al. 2008. Effects of offshore platforms on soft-bottom macro-benthic assemblages: a case study in a Mediterranean gas field. *Marine Pollution Bulletin*, 56: 1303–1309.
- Vandendriessche, S., Derweduwén, J., and Hostens, K. 2015. Equivocal effects of offshore wind farms in Belgium on soft substrate epibenthos and fish assemblages. *Hydrobiologia*, 756: 19–35.
- Vanhellemont, Q., and Ruddick, K. 2014. Turbid wakes associated with offshore wind turbines observed with Landsat 8. *Remote Sensing of Environment*, 145: 105–115.

- Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73: 5261–5267.
- Whomersley, P. P., and Picken, G. B. O. 2003. Long-term dynamics of fouling communities found on off-shore installations in the North Sea. *Journal of Marine Biological Association UK*, 83: 897–901.
- WoRMS—World Register of Marine Species—Phoronidae Hatschek, 1880 (n.d.). <http://www.marinespecies.org/aphia.php?p=taxdetails&id=148378> (last accessed 27 February 2018).

Handling editor: Silvana Birchenough