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Benthic Indicator Species Index (BISI)

Development process and description of the National Benthos Indicator North Sea including a protocol for application

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Photos front left top to bottom right: a) Pagurus bernhardus (common hermit crab) a typical and smart species for the Voordelta, the Vlake van de Raan and the North Sea Coastal Zone (H1110b) and a typical species for the Dogger Bank (H1110c); b) Alcyonium digitatum (dead man's fingers soft coral) a typical and smart species for the Cleaver Bank (H1170); c) Goneplax rhomboides (angular crab) a smart species for the Frisian Front; d) Neptunea antiqua (red whelk) a typical species for the Dogger Bank (H1110c). All animals caught during the International Beam Trawl Survey (IBTS) on the international North Sea in 2008. Photos O.G. Bos, from Wageningen University & Research - Image Collections.

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Abstract

In this report, the process of development, the methodology and the application of the Benthic Indicator Species Index (BISI) is presented as a national benthos indicator for the Dutch North Sea. The current report comes with a protocol for application (provided as an appendix), and a BISI assessment tool in Excel.

The national benthos indicator BISI was developed during 2016-2017 for the evaluation of habitat quality, seafloor integrity and the ecological functioning of the benthic fauna for the Marine Strategy Framework Directive (MSFD). Data are retrieved from the MSFD benthos monitoring programme. Evaluations of the effectiveness of fisheries measures in areas with special ecological values (ASEVs) can also be carried out with the BISI. In addition, the methodology has been developed to support quality assessments in the context of reports on the Habitats Directive habitat types to the European Commission and evaluations of the conservation objectives of marine habitat types in Natura 2000 management plans.

This report describes the process of realization, the choices made, and present the methodology in a protocol. In addition, the application is explained and an Excel file is included to calculate BISI scores and present the first results of observational data.

The BISI indicator compares the occurrence (or spatial detection probability) and / or densities (n / m^2) of a selection of indicator species at a given moment with a reference condition. The reference and the indicator species list are area-specific. The method consists of calculating a (geometric) average of the weighted log-transformed observation-reference ratios, for which results are back-transformed afterwards.

With statistical testing of potential differences, the variances as present in the observation data are taken into account. The reference value is by definition 1. For each indicator species, the value can vary in a range of a factor by hundred be as high as 100 times the reference value, or a hundredth fraction of the value. When the values of the observations exceed this range, the values are being normalized to the minimum and maximum values of the range in order to cope with 'zero' values and to minimize the effects of rare or abundant species that else might dominate the assessment.

The BISI indicator calculates an index value for the general quality status of an area based on a set of indicator species. In addition, the indicator calculates specific BISI values based on a subset of indicator species to indicate the possible causes and the consequences of observed changes in the quality condition of the benthos.

The Benthic Indicator Species Index is calculated as follows:

$$BISI = \exp((1/S) \sum (IV_i) \log(O_i/R_i)),$$

At which:

S = the number of indicator species in the evaluation of the relevant area,

IV_i = the species-specific indicator value (value determined for each specific assessment),

O_i = observed abundance of species i at the time of evaluation,

R_i = reference abundance for species i (internal reference).

The BISI is calculated in this report for the following applications:

- Habitat quality in Natura 2000 / MSFD areas: Dogger Bank, Central Oystergrounds, Frisian Front, Cleaver Bank, North Sea Coastal Zone, Voordelta (Front Delta), Vlakte van de Raan (Plain of the Raan). Supplemented with the Brown Bank, an area representative for the Southern Bight.

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- Habitat quality of national Habitats Directive Annex 1 habitat types: H1170 (Reefs), and H1110 (Sandbanks slightly covered by sea water all time, divided into H1110b – Sandbanks slightly covered by sea water all time in the coastal zone, and H1110c – Sandbanks slightly covered by sea water all time in the offshore).
- Habitat quality of EUNIS ecotopes at classification level 3: Deep coarse sediment -, Deep mud -, Deep sand -, Shallow to moderately coarse sediment -, Shallow to moderately deep sand -, and Shallow to moderately deep mud habitat.
- Effect of fisheries measures in Natura 2000 / MSFD areas: Comparison of closed areas with open areas.

Indicator species

Indicator species are species for which fluctuations in their occurrence (densities and / or spatial distribution) are considered indicative for the quality of the living environment (the degree and type of pressure on the system that is present). Species are assessed as indicative if they are sensitive to a specific pressure, if natural fluctuations are relatively small compared to fluctuations under the influence of a specific pressure, and if the species can be detected in natural densities using conventional observation techniques and relatively low monitoring efforts. The 'smart' species selected at an earlier stage (species with the above characteristics that have been observed in reasonable numbers recently, Wijnhoven et al., 2013) and the typical species of the Habitats Directive are part of the specific selections of indicator species. Also, species that have not been observed recently, have been selected when there are indications that they can be present in the area concerned and can return in a natural way when the quality status is good (their absence is indicative for the current quality status). The reliability of the assessment methodology increases when a larger number of species is included in the assessment. As a criterion for the selection of 'smart' species, the composite score of several sensitivity aspects was calculated (Wijnhoven et al., 2013). Since a sensitivity to only one aspect (e.g. known sensitivity to seabed disturbance or known sensitivity to eutrophication) is already sufficient to be indicative, and will actually help in the indication of the cause of a certain quality state, also those species (additional indicator species) are included in the list of selected indicator species.

The methodology is in line with and adapted to the current (and expected to be consistently applied the coming years) MSFD benthos monitoring programme. This programme consists of sampling with boxcorer, dredge (in coastal areas sometimes replaced by suction corer or Van Veen grab) and on the Cleaver Bank mainly Hamon-grab samples and video transect inventories. An important factor is the sampling scheme, whether sampling locations are initially assigned randomly over an area (and thus provide a more or less representative image of the total state) or whether these are randomly stratified allocated and then are specifically intended for the evaluation of the area or habitat concerned (mainly used for the assessment of the effects of management measures).

1 Introduction

With the elaboration of the first part of the Marine Strategy for the Dutch part of the North Sea for the period 2012-2020 (Min IenM & Min ELenI, 2012), a route has been set out towards a good environmental status of the marine environment in 2020. In addition to the characterization of the system at that time on the basis of the information available in 2012 (Initial Assessment), the good environmental status was defined and targets and indicators were drawn up. The macrozoobenthos (the invertebrates living in and on the bottom) of the North Sea play an important role in the Marine Strategy. The benthos significantly contributes to the biodiversity, several species (especially some shellfish) are of commercial importance, benthos plays an important role in the marine food chains, and contributes significantly to the structure and function of the seabed and the underwater ecosystems in general. Conservation of biodiversity of the benthos is an objective in itself under the Marine Strategy Framework Directive; densities, condition of populations and spatial distribution of long-living and (for physical seabed disturbance) vulnerable species must be improved. Furthermore, the composition of the benthos is indicative of the quality status of habitat types from Appendix I of the Habitats Directive (H1110: Sandbanks slightly covered by sea water all time, H1170: Reefs), for which conservation and / or improvement objectives have been formulated. In addition, the benthos is also indicative for various other environmental objectives (such as an indication for aspects such as eutrophication, hydrographic properties and pollutants status, and the presence of various exotics among the benthos) that are not specifically addressed here.

The important role that benthos plays in the underwater system and thus also in the Marine Strategy meant that a corresponding monitoring program had to be drawn up, on the basis of which the quality situation and developments in that situation can be monitored and assessed. In order to arrive at a monitoring program that meets the data needs, a study to support the selection of indicator species was conducted in 2013, Wijnhoven et al. (2013). In addition, monitoring scenarios have been developed for the various areas for which information is desired, with considerations between efforts and numbers of species of which developments can be evaluated in the short and medium-long term. This resulted in the choice for the current National Benthos Monitoring Programme for the North Sea (MSFD monitoring programme benthos: Min IenM & Min EZ, 2014). To this day, there are adaptations and actualizations to the monitoring programme: Troost et al. (2013), Van Kooten (2013), Van Asch & Troost (2014)), which meet the needs of evaluating the effectiveness of management measures taken. These largely concern the monitoring of closed areas for seabed-disturbing fisheries, compared to open areas for such fisheries, for which the programme is generally adapted as soon as measures have taken shape.

Based on the MSFD benthos monitoring programme, a National Benthos Indicator for the North Sea has been developed as described in this report. The report by Wijnhoven et al. (2013) has been the starting point. Specifically, the demand from the clients has been to develop an integrated indicator, in which different indicator species are combined. The indicator had to provide insight into the quality situation and development of areas and sub-areas (areas with special ecological values, EUNIS ecotopes, Habitats Directive habitats and areas with specific management measures) of the Dutch North Sea. It is important that it can be demonstrated whether the quality based on the benthos composition is unchanged, or whether it increases or decreases. Specifically, it should be able to use the National Benthos Indicator for assessments and reports for the Marine Strategy Framework Directive (MSFD), the Habitats Directive (HD), the evaluation of the Natura 2000 management plans and the evaluation of the effectiveness of the management measures taken. This means that different spatial and temporal scales play a role and that the indicator should be able to deal with this. In addition, the indicator must also be relatively simple and transparent, so that outcomes can be clearly and easily interpreted by users (policy-makers and managers) and communicable to stakeholders.

The current report presents the National Benthos Indicator for the North Sea, the Benthic Indicator Species Index (BISI), specifically (but not exclusively) developed for the evaluation of the quality status of the Dutch part of the North Sea. The report discusses the process of developing the

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indicator and the backgrounds and choices made. The indicator is described in a protocol for application (Annex 1) to facilitate testing and implementation of the index by others.

Table 1. Glossary.

Term	Meaning
Additional indicator species	Indicator species that have been added to the area-specific selections of 'smart species' and 'typical species'. Where 'smart species' are potentially the most suitable indicator species for the indication of general quality changes at short term, species that are specifically indicative of one quality aspect and / or currently rare or not found (but known to have recently been present), may be valuable for the identification of causes and consequences of quality changes.
ASEV	Area with Special Ecological Value: Areas with special ecological values, as mentioned in Lindeboom et al. (2005). Areas with special ecological values (ASEVs) have been designated as representative areas within the MSFD benthos monitoring programme, which comprise the most important ecotopes and habitats of the Dutch North Sea. The areas have also been called 'MSFD areas' in the past (Wijnhoven et al., 2013). The monitoring and evaluation of the NCP is therefore focused on the ASEVs. This concerns the HD areas of the North Sea, the Frisian Front and the Central Oystergrounds (together representative of the Oystergrounds) and the Brown Bank (representative of the Southern Bight).
BD	Birds Directive
CFP	Common Fisheries Policy
BISI	Benthic Indicator Species Index
Closed areas	Designated management areas where (specific) seabed-disturbing fishing activities are prohibited. Sub-areas of or near 'areas with special ecological values' (ASEVs) are assigned in the frame of VIBEG (Fisheries Protected Areas) or FIM-PAS (Fisheries Measures in Protected Areas) to improve the quality of the underwater environment
EC	European Commission
Ecotope	EUNIS level 3 ecotope (referred to as 'habitat' in the EUNIS system)
H1110	Habitats Directive habitat type Sandbanks slightly covered by sea water all time ', with the Dutch subtypes: H1110a: Sandbanks slightly covered by sea water all time with strong tidal influence (Wadden Sea and mouth of the Haringvliet); H1110b: Sandbanks slightly covered by sea water all time in the North Sea coastal zone; H1110c: Sandbanks slightly covered by sea water all time in the vicinity of the Dogger Bank.
H1170	Habitats Directive habitat type 'Reefs'

Habitat type	Habitat types as listed in Annex 1 of the Habitats Directive
HD	Habitats Directive
Indicator species	Species with specific indicator value. A significant change in the abundance of indicator species is indicative of a certain quality change for the area to be evaluated. Species are suitable as indicator species when they are sensitive to specific changes in pressures and / or represent specific ecological values, where populations can be monitored using standard techniques. The BISI uses area-specific evaluations based on the occurrence of indicator species where the list of indicator species concerns both 'smart species' and 'typical species' and a number of additional species with indicator value.
Indicator value	The extent to which change in abundance of a species is indicative for changes in the general quality state and / or specific causes or consequences of observed changes in quality. In the BISI the (specific) indicator value is always a value between 0 (not indicative) and 1 (very indicative) and is presented in the BISI formula with IV (Indicator Value).
Internal reference	Area-specific composite realistic reference with regard to the abundance of benthic indicator species (which means that when the prevailing pressures are largely removed the described situation can be reached naturally given the current habitat constitution and available species pools) used in the BISI methodology to compare the quality status of a period to be evaluated with.
MSFD	Marine Strategy Framework Directive
MSFD-areas	Expression used in the past (Wijnhoven et al., 2013) for Areas with Special Ecological Values (ASEVs).
National Benthos Indicator	Synonym of BISI as used in the Netherlands
NCP	The Dutch Continental Shelf (NCP) is the same as the Dutch Exclusive Economic Zone (EEZ).
'Smart species'	In Wijnhoven et al. (2013), potential indicator species have been referred to as 'smart species' when, for an area with special ecological values, they have achieved a high score for a composite formula that shows the degree of sensitivity to a number of pressures, the representativeness for ecological aspects and the recent presence and detectability.
Typical species	Initial lists of species presented in LNV (2008) and Paijmans & Asjes (2012) that under natural conditions do not show large population fluctuations, and are considered indicative for a good abiotic status (Ca), indicative for a good biotic structure (Cb), indicative for a good abiotic state and good biotic structure (Cab), characteristic species (K) and exclusive species (E), as identified for the Habitats Directive habitat types. Lists with typical species for H1110 and H1170 were actualized in 2014 (Min EZ, 2014a,b).

2 Preconditions and starting material

The National Benthos Indicator / Benthic Indicator Species Index (BISI) has been developed specifically for the evaluation of the Good Environmental Status (GES) under the MSFD and the developments in the quality status of the Dutch North Sea based on the benthic species composition. The indicator can be used for assessing the quality of benthic communities (in terms of biodiversity of the benthos and quality of seabed). This can be done at different levels: (1) within MSFD areas (also called Areas with Special Ecological Values (ASEVs)) and Natura 2000 areas; (2) at the level of EUNIS ecotopes level 3; (3) for the national assessment of Habitat Directive Annex 1 habitat types (4) and to determine differences between open and closed areas for seafloor disturbing fisheries. Figure 1 provides an overview of the various evaluations for which the National Benthos Indicator is deployed or can be used.

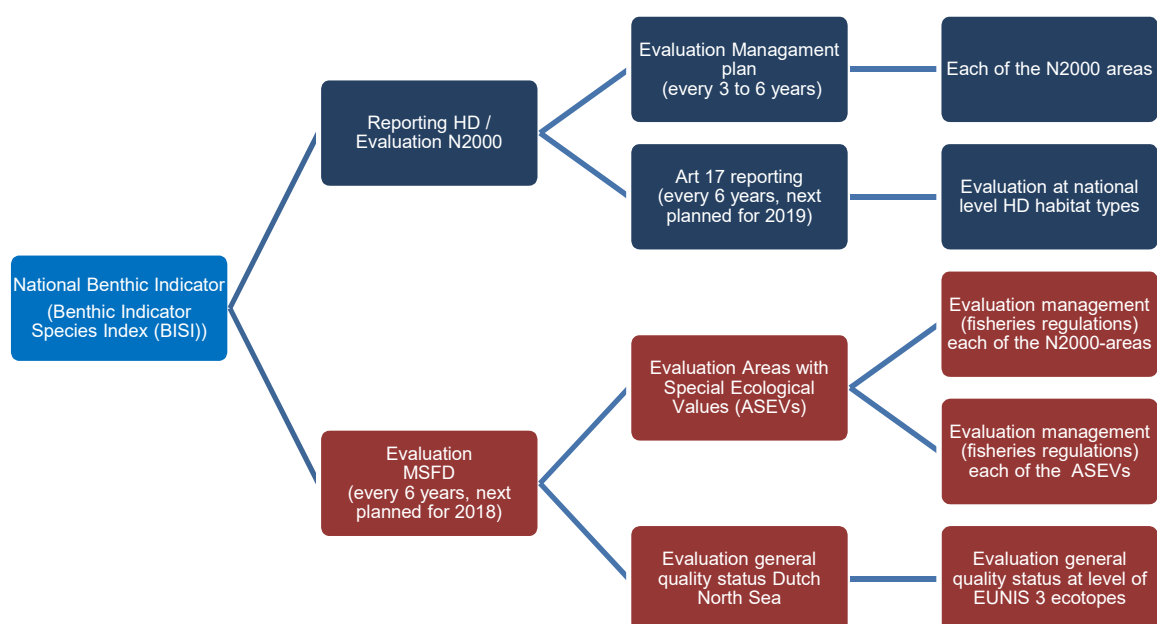


Figure 1 Schematic overview of the different Dutch evaluations for which the Benthic Indicator Species Index (BISI) is developed.

Focus on Areas with Special Ecological Values (ASEVs):

Areas with special ecological values

The areas with special ecological values (ASEVs) were initially identified in Lindeboom et al. (2005), and later also referred to as MSFD areas (including Wijnhoven et al., 2013). The ASEVs are assumed to be representative for specific components and nature values as present on the Dutch Continental Shelf. The MSFD benthos monitoring programme, and with it the evaluation, is therefore focused on the ASEVs. The ASEVs have often acted as initial search areas for management measures (although recently areas closed for seafloor disturbing fisheries have been designated partially or almost entirely outside the ASEVs) and include all HD and also all BD areas of the NCP.

Where the MSFD calls for an evaluation of the general quality status of the Dutch North Sea and the benthic 'habitats' and benthic communities in particular, the Netherlands has chosen to achieve

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conservation and improvement of the seafloor environment by focusing on a number of Areas with Special Ecological Values.

The ASEVs are in terms of ecotope composition often representative for larger units within the Dutch North Sea and / or cover specific 'habitats'. The use of ASEVs is thus in fact a way of maintaining and / or improving the overall quality of the North Sea. Since the Dutch North Sea (like most marine systems) consists of different ecological units with their own characteristics – expressed in, amongst others, the benthic communities and, for example, the vulnerability to various types of disturbances – the evaluation is split up at marine ecotope level. Within an ASEV multiple ecotopes can be present.

Subdivision in ecotopes (EUNIS 3):

For classification into ecotopes, the EUNIS (European Nature Information System) classification at level 3 (v2016) is used (as available via www.emodnet-seabedhabitats.eu and elaborated by the European Environment Agency (EEA)). The EEA itself speaks about seafloor habitats instead of ecotopes. When using a further subdivision at EUNIS level 4-6, these may be regarded as habitats. However, when working with a more general classification in only a few classes for the entire Dutch North Sea, the term 'ecotope' is more appropriate. The term 'ecotopes' of the MSFD also prevents confusion with the term 'habitat types' of the Habitats Directive. There are considerable differences between the EUNIS ecotope maps from the past and the version as adopted (v2016), since a different classification has been used. It has been decided to distinguish 6 classes, of which the quality status and quality development will be evaluated separately (see also Figure 2).

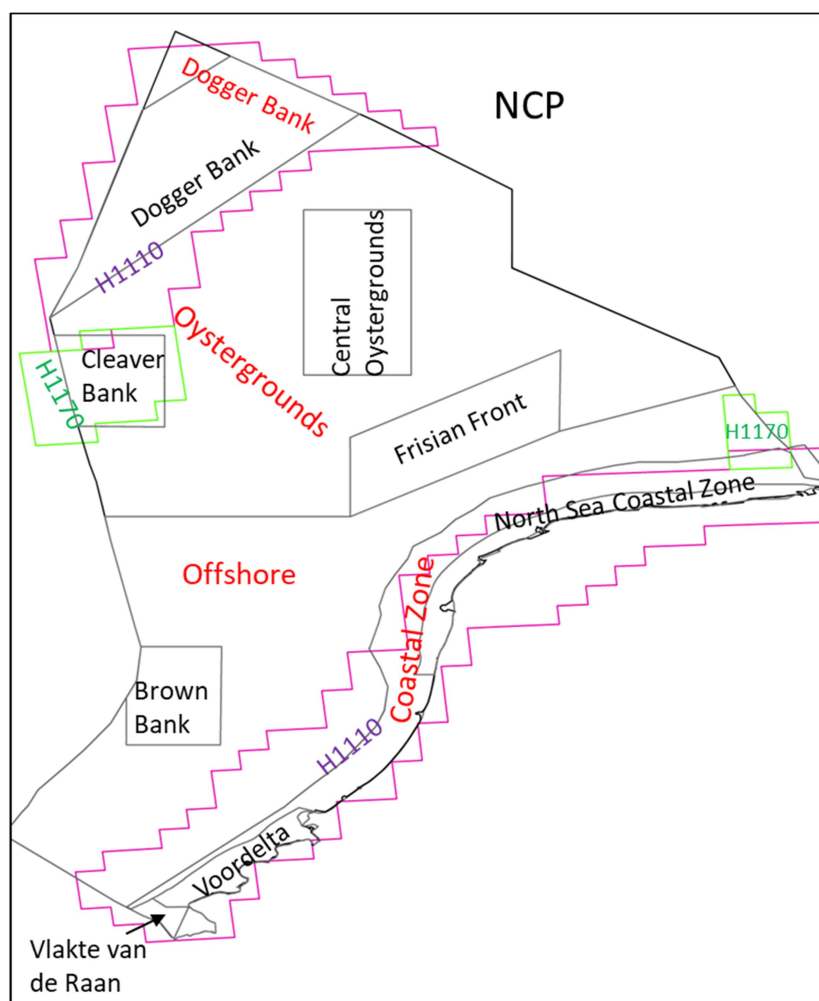


Figure 2a. Map of the Dutch North Sea and environs with indication of the Dutch Continental Shelf (NCP) and the subdivision into 4 MSFD zones (names in red). Areas with Special Ecological Values (ASEVs) are indicated by gray contours (names in black), grid areas (10x10 km) in which Habitat Directive habitat types H1110 and H1170 (situation 2007-2012: Min EZ, 2013) are identified with pink and green contours (and codes) respectively.

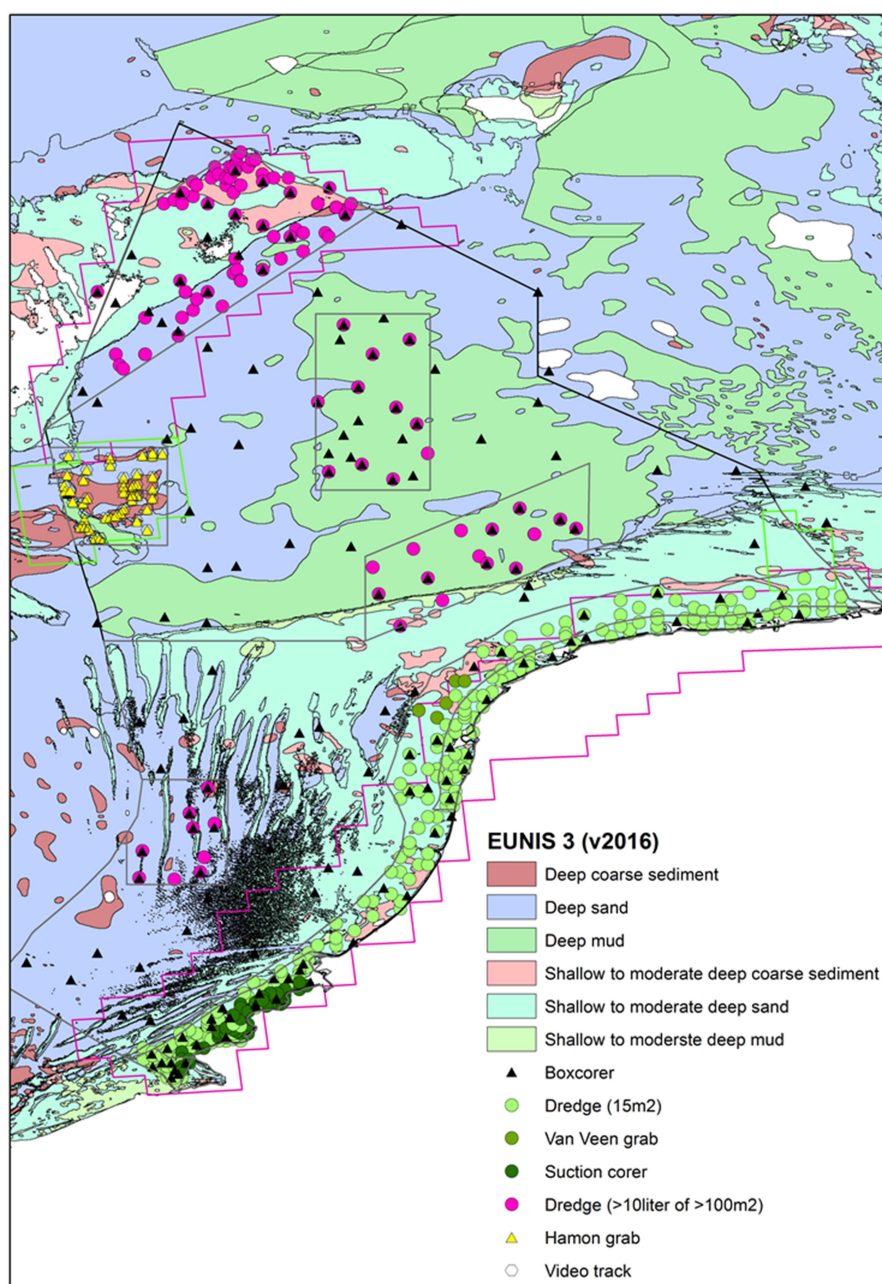


Figure 2b. A map of the Dutch Continental Shelf (NCP) in which the sample locations that are part of the MSFD benthos monitoring programme are indicated (including sampling methodology used) are indicated. The background map shows the division into 6 ecotope types (classification at EUNIS level 3, v2016, available via: www.emodnet-seabedhabitats.eu) that are part of the MSFD evaluation. (Areas with restrictions to seafloor disturbing fisheries that are part of the assessments under the MSFD are not shown as they are not all definitive yet).

It concerns the categories:

- A5.15: Deep coarse sediment,
- A5.27: Deep sand,
- A5.37: Deep mud,
- A5.14: Shallow to moderate deep coarse sediment,
- A5.25, A5.26: Shallow to moderate deep sand, and
- A5.35, A5.36: Shallow to moderate deep mud.

The EUNIS 3 classification makes a distinction in several subtypes, for example 'Shallow to moderately deep fine sand' (A5.25), 'Shallow to moderately deep muddy sand' (A5.26) and 'Shallow to moderately deep mixed fine - and muddy sand' (A5.25 or A5.26) which has been merged into 'Shallow to moderately deep sand'. It appears that in practice the classification at EUNIS 3 level is not completely covering the area of the NCP. There remain some small areas that are designated at

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EUNIS 4 level as 'Moderate energy deep circalittoral seabed'. As the expectation is that moderate hydrodynamics indicates the presence of sand rather than mud, it has been decided to classify these areas as 'Deep sand' ecotope for the evaluation (in practice, however, no problems are expected with regard to the classification of those areas, because there are as yet no sample locations in it).

Distinguish areas inside and outside ASEVs:

In addition to assessment covering the complete area of the NCP based on the quality of the EUNIS ecotopes, the quality status of individual ASEVs is evaluated because at this level often specific measures are (being) taken and the first improvements are expected, while the monitoring also focuses on these areas (selected because of their representativeness of the habitats included). Due to the known differences in monitoring efforts and measures to be taken within ASEVs (and generally not outside of them), it is necessary to first evaluate the parts inside and outside ASEVs separately for the evaluation of EUNIS ecotopes at the NCP level. In addition to the evaluation of the quality status of the individual ASEVs as a whole, a specific evaluation of the management measures will be performed. This concerns restrictive measures with regard to seafloor disturbing fisheries. Designated areas where (specific types of) seafloor disturbing fisheries is excluded are hereinafter referred to as 'closed areas'. Per ASEV (which can also have a status as Natura 2000 area), the quality development in closed - relative to open areas is evaluated. This is done on the basis of selected samples so that sampling effort and sampled ecotope or sampled initial community are comparable within and outside the closed areas, which enhances the expressiveness (power) of the statistical evaluation.

Possible distinction between closed areas:

For certain ASEVs (such as on the Dogger Bank) it has been decided to evaluate closed areas (Northern and Southern slopes) separately, because the communities present show large differences (and the expressiveness of the analyses does not benefit from merging the areas into one cluster. In other ASEVs, 'closed areas', with different management measures regarding fisheries types restricted, are merged in the evaluations because it concerns quite fragmented situations, for which the required number of samples would increase considerably to distinguish the expected nuances in management and quality changes compared with separate evaluations. The required monitoring effort is thus reduced as much as possible, whereby splitting leads to two analyzes with a required number of samples, but the smaller variation in benthic species composition within the split areas requires a smaller number of samples to achieve the same power for the tests.

MSFD quality objectives:

Apart from the fact that the quality status and quality development of the various (partially subdivided) areas can be evaluated using the method presented here, objectives have also been formulated from the MSFD with regard to the result of the evaluations (Table 2). For all listed ASEVs with the exception of the Brown Bank (7 areas) and the EUNIS ecotopes at national level (6 classes), good environmental status is defined as at least a maintaining the present level of the quality of the 'habitats' and on the medium-long term (as a result of management measures) an improvement of quality. This means that the evaluations should not show a significant decrease compared to the T0 and should show a significant increase over time (indicative 2 to 3 MSFD cycles corresponding to 12 to 18 years) (Min lenM & Min EZ), in prep.). In the case of the 'Deep sand' ecotope, this is in principle formulated for the MSFD as 'Deeper non-dynamic sandy soils'.

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Table 2. Overview of the quality objectives for which the Benthic Indicator Species Index (BISI) is used, taking into account the update of the Marine Strategy planned for 2018 (Min IenM & Min EZ, in prep).

	Unit	Minimum criterium	Medium-long Term	
Marine Strategy Framework Directive (MSFD):				MSFD and the Common Fisheries Policy (CFP):
Areas with special ecological value (ASEVs)	Dogger Bank	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open
	Cleaver Bank	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open
	Central Oystergrounds	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open
	Frisian Front	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open
	North Sea Coastal Zone	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open
	Voordelta	No sign. decrease BISI	Sign. increase BISI	Sign. increase quality ^A closed cf. open
	Vlakte van de Raan	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open
Ecotopes (EUNIS level 3) at national level	A5.15: Deep coarse sediment	No sign. decrease BISI		
	A5.27: Deep sand ^B	No sign. decrease BISI		
	A5.37: Deep mud,	No sign. decrease BISI		
	A5.14: Shallow to moderate deep coarse sediment	No sign. decrease BISI		
	A5.25, A5.26: Shallow to moderate deep sand	No sign. decrease BISI		
	A5.35, A5.36: Shallow to moderate deep mud)	No sign. decrease BISI		
Habitats Directive (HD) and Evaluation management Natura 2000 areas:				
Natura 2000 areas	Dogger Bank	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open
	Cleaver Bank	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open
	North Sea Coastal Zone	No sign. decrease BISI	Sign. increase BISI	Sign. increase BISI closed cf. open

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	Front Delta	No sign. decrease BISI		No sign. decrease BISI closed cf. open
	Vlakte van de Raan	No sign. decrease BISI		No sign. decrease BISI closed cf. open
Habitats Directive (HD):				
Habitats Directive- habitats at national level	H1110 (Sandbanks slightly covered by sea water all time) ^{C,D}	No decrease number of typical species		
	H1170 (Reefs) ^C	No decrease number of typical species		

^A BISI is not used for the time being because the Voordelta has its own assessment system with special monitoring (Craeymeersch et al., 2015), and a goal of 10% increase in biomass for which testing based on the BISI is not necessary.

^B The MSFD specifically refers to 'deep low-dynamic sand'. The quality assessment on the basis of the BISI will be positive if the share of 'low-dynamic sand' compared to 'high-dynamic sand' increases or decreases, if other pressure factors are constant. However, in addition to the BISI, the total surface area of 'low-dynamic sand' cf 'high-dynamic sand' must be included in the evaluation.

^C For the time being, only the number of typical species of the Habitat Directive habitat is part of the quality assessment. The BISI could, however, provide background information on the causes of any quality changes and could give signals on the direction of the changes in quality of the habitats. A specific evaluation based solely on typical species is a standard part of the current BISI to meet the wishes regarding Habitat Directive evaluations.

^D In view of the partly different conditions with corresponding species composition on the offshore sandbanks slightly covered by sea water all time, and sandbanks slightly covered by sea water all time located in the coastal zone, H1110c and H1110b are separately evaluated on the basis of the BISI (H1110a, Sandbanks slightly covered by sea water all time in areas with strong tidal influence), is largely located outside the NCP and not (yet) developed in the current BISI.

In the event of an increase in the overall (average) hydrodynamics in the deep sandy ecotope or an increase in the surface area of high dynamic area within the sandy ecotope, the assessment will also show deterioration in the quality situation as various indicator species are sensitive to an increase in the hydrodynamics. BISI results should therefore be evaluated for assessment together with the surface development of individual EUNIS ecotopes, as an increase in highly dynamic sandy ecotope is not necessarily bad when it is not at the cost of the low dynamic ecotope. In addition, the fisheries measures are being evaluated under the MSFD and the Common Fisheries Policy. Of course, in the long term, the quality in the closed areas will be expected to improve compared with the development of the open areas. The result for the entire ASEV should then of course be an improvement in the quality situation as described above in the medium-long term. In addition to evaluating conservation and / or improvement of the seafloor as habitat (MSFD D6C3), the quality of benthic communities is specifically evaluated on the basis of the size, condition and spatial distribution of populations. In practice, we have the same indicator species with regard to the National Benthos Indicator. Habitats and benthic animals are connected; it is at most a different cause or pressure factor whose effects become visible in the same indicator because either the habitats (and therefore indirectly the benthic animals) or the benthic animals (and therefore indirectly the habitats) are being influenced. With regard to the specific benthic species (the set of indicator species per area) the objective is that their occurrence (to be measured by the development of the BISI as for the habitat objectives) will show a significant increase in the closed compared to the open areas (this concerns all aforementioned ASEVs where measures are or will be taken in the near future).

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The Brown Bank, which has more intensive monitoring as (representative) ASEV, has an exceptional position as ASEV because for this area no specific objectives have been drawn up yet. For the time being, the method presented here is not used for the evaluation of the effectiveness of the fishing measures in the Voordelta, because here a specific assessment program has been running for years with tailored design (Craeymeersch et al., 2015). The MSFD benthos monitoring is therefore not specifically geared to the evaluation of management measures in the Voordelta.

Natura 2000 and HD objectives:

The assessment of Natura 2000 conservation objectives for habitat types is described in the relevant so-called profiles such as H1110 (Min EZ, 2014a) and H1170 (Min EZ, 2014b). In principle, evaluation is based on four sub-aspects (as usual for terrestrial habitats). However, marine habitat types are essentially different from the terrestrial habitat types, and are somewhat difficult to grasp in the four "pillars". However, there is a strong link with the MSFD: The objectives of MSFD and N2000 largely correspond with respect to the content (see also Table 2). The monitoring program that is set up for the MSFD can therefore be used one-to-one for the N2000 monitoring plans. Because goals are the same, it is logical and perhaps desirable to harmonize the assessments. Conversely, the typical species of the marine habitat types have already been taken into account in the development of the benthos indicator BISI. The method presented here has been developed, by already integrating all the available typical (benthos) species of the marine habitat types H1110 and H1170 (Min EZ, 2014a,b) into the methodology (including a standard procedure for the special assessment of the HD typical species for the area to be evaluated). According to the Natura 2000 system, an evaluation of the management plan for the Natura 2000 areas is carried out every 6 years (with an optional interim evaluation) (Table 2). For the areas designated for the Habitats Directive (Vlakte van de Raan, Voordelta, North Sea Coastal Zone, Dogger Bank and Cleaver Bank), holds that they are also ASEVs for which the quality of the seafloor and the benthic communities under the MSFD is evaluated, including an evaluation of the measures taken. New data are available every 3 years and a complete evaluation can be carried out, whereby the requested moment of reporting determines which monitoring years can be included.

For the Article 17 report that evaluates the quality status of the HD habitat types at national level (see Figure 1), a specific assessment had to be made in the Netherlands. For habitat type H1110 (Sandbanks slightly covered by sea water all time), the assessment had to be differentiated for two sub-types. There are separate assessments for H1110c (Sandbanks slightly covered by sea water all time in the vicinity of the Dogger Bank) and H1110b (Sandbanks slightly covered by sea water all time of the coastal zone) because the communities are partly different, and merging in one evaluation brings great uncertainties. The H1110b habitat type in particular comprises several EUNIS ecotopes and the ASEVs North Sea Coastal Zone, Voordelta and Vlakte van de Raan, so that consequently the established reference has become a combination of the mentioned ASEVs. For the derivation of the reference values, in addition to the values for the individual ASEVs, the weighted average of the 3 ASEVs was taken into account. Habitat type H1110a has been left out of consideration for the time being, because this mainly concerns the Wadden Sea which is not part of the National Benthos Indicator for the North Sea. (Of course, a similar method could be developed for transitional waters with a relative extensive benthos monitoring program, such as the Wadden Sea. The results of the Cleaver Bank assessment can also be used as an assessment of habitat type H1170 (as long as there is no benthos sampling in the area of the 'Borkum Stones' site).

The relevant quality objectives following from the Habitats Directive concern the maintenance of the quality of the seafloor (the habitat types present) in accordance with the management plans for the Voordelta and the Vlakte van de Raan, and improvement of the quality of the Natura 2000 areas North Sea Coastal Zone, Dogger Bank and Cleaver Bank.

The quality of habitat types H1170 and H1110 is, apart from the development of the surface, determined by, among other things, the assumed presence of typical species. The method developed here could provide insight into the development of the populations of the group of typical species

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within the habitat types so that signals can be picked up of which direction the development of the quality of the HD habitat types is heading towards.

Evaluation based on indicator species:

Already in 2013 a way was taken to evaluate the quality situation and developments of the NCP in the future, with the selection of a number of potential indicator species (at the time called smart and typical species) (Wijnhoven et al., 2013). The results from the study, with a first selection of indicator species and the appreciation of their indicator value, based on a broad literature study with input from various experts, were taken as the starting point for the development of the evaluation methodology. The indication value was determined on the basis of a fixed formula in which scores were combined related to 1) recent occurrence, 2) being characteristic for the area to be evaluated, 3) sensitivity to seafloor disturbance, 4) sensitivity to ecological disturbance, 5) importance for ecological processes, 6) potential size, 7) indicative for early stages of recovery, 8) potential age. Since the current MSFD benthos monitoring programme is also designed to detect changes in abundance and / or the spatial occurrence of the selected species, we did not repeat the assessments at the time, but at most evaluated individual cases again. We have, however, estimated the indicator value of a number of new / additional species.

Characteristics of available data:

For the development of a methodology, we are of course dependent on the availability of data. This means that for the derivation of references we are dependent on the data density from the past and the specifications of the sampling techniques that have been used at the time. For some parts of the North Sea, the sampling density has not been very high in the past, which of course provides fewer means for the development of a reliable methodology specific to that area. We have compensated this largely by using other sources (historical data and observations from data sets not supplied directly by the client: see overview in chapter 3.5). Other characteristics of available monitoring data of which we were dependent on development are: variable sampling surface, subsampling and / or not recording all species of the dredge samples in the past, by which the evaluation of spatial detection probability based on dredge data is not an option.

The above also has consequences for the observation of at present less common species. Especially for some typical species this may mean that they are missed while they may be present. The expectation is, however, that this chance of missing is dependent on the abundance. This means, among other things, that the methodology will be more suitable for evaluating relative differences in quality over time (or between areas according to a pre-set design) than that the quality state of different areas can be directly compared.

The use of different techniques, such as the dredge, Van Veen grab and suction corer, has consequences for the observation of species. As the monitoring programme for the coming years has been established and the same locations will be visited using the same techniques according to the monitoring programme, it is expected that the probability of observing species (assuming the abundance remains the same) will not change substantially.

Methodology extremely suitable for detecting quality changes:

The methodology has been developed specifically for being able to detect quality changes. At the moment we only have a complete set of data available for the methodology for the year 2015. This means that we have not yet been able to test the methodology optimally in all aspects. Although we do not immediately expect adjustments to be necessary in the short term, it is precisely the assessment of one point in time (not the strongest point of the method) that can give some uncertainties. It is especially the evaluation of relative quality differences between areas on the T0 that is sensitive and dependent on the internal reference used. We are, however, convinced that the internal reference, based on various datasets and working with a range of indicator species, will work well. An evaluation of the methodology on that point, based on the results after a few monitoring rounds, is however recommended.

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Required sampling effort:

During the development of the methodology, we assumed that the MSFD monitoring programme for benthos that was implemented in 2015 will be maintained in the near future. Reducing the number of sampling sites and / or changing methodologies can have consequences for the applicability of the methodology and the reliability of the evaluations. This does not mean, however, that the methodology is rigid. The National Benthos Indicator according to the BISI is especially suitable for dealing with changes in, for example, sampling efforts. However, it should be considered whether the internal reference for the new situation suffices and whether the desired changes can still be detected on the basis of the new situation.

3 Development process

The development of a well-functioning National Benthos Indicator for the North Sea that should be able to be used for a long period for various reports and evaluations, to be applied considering a large number of (sub) areas and spatial scales as elaborated earlier under the 'Preconditions and basic material', that must be transparent and communicable, is a complicated matter that requires a step-by-step approach and the use of different sources. The development was therefore a (gradual) process in which choices were made on the basis of findings and / or consultation of experts and sources. The following steps have been taken (not necessarily in chronological order, as findings and / or insights were often a reason to return to previous steps or to first (partially) take other steps).

3.1 *Alignment with existing initiatives and learning from international exercises*

Based on the reports and findings of Wijnhoven et al. (2013), which were already based on extensive literature review and expert consultations, we have of course also learned from various other initiatives related to benthos indicator development. The following initiatives should certainly be mentioned:

Specific sensitivities of different types:

The findings from projects such as BENTHIS and DEVOTES (Rijnsdorp et al., 2016, Beauchard et al., 2017) that are currently being developed within ICES clearly show that species show different sensitivities for different types of disturbances and can therefore have an important indicator value. In both studies the link is made to specific characteristics of species (often called 'traits'). Among others, the studies point to the fact that differences in susceptibility of benthic communities exist between different habitats (this means that a certain pressure might have more impact in one habitat than in another). In addition, it should be taken into account that benthic communities of frequently disturbed environments may consist predominantly of stress-adapted (resistant) species, so that the removal of a certain pressure does not have to lead directly to a change in the benthic faunal composition (Duineveld et al. , 2007).

Knowledge exchange took place with both Olivier Beauchard (DEVOTES and spin-off activities) and GerJan Piet (BENTHIS). The characterization on the basis of 'traits' according to Beauchard (Beauchard (2016), largely based on the work of Greenslade (1983) and Southwood (1988)) has been compared with the Indicator Value (IV), allocated to potential indicator species in BISI (see Appendix 3). When r-strategists (resilient species) and A-strategists (resistant species) according to Beauchard, had an IV of 1 for seafloor disturbance in our initial assessment, they were adjusted to 0.5. When K2 strategists (highly sensitive species) and K1 strategists (susceptible species) had an IV of 0, they were also adjusted to 0.5. In addition, extra attention has been paid to ensuring that indicator species are specific to areas / ecotopes. The findings in BENTHIS and DEVOTES also support the importance of specific evaluations in addition to the general quality assessment for the

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identification of causes and effects; and the importance of involving multiple species in evaluations for a reliable result.

Technical aspects of the methodology:

Parallel to the National Benthos Indicator development, trend analyzes were carried out by Statistics Netherlands (CBS) in the context of the Living Planet Index (LPI) (Poot, 2016). With regard to the benthos of the North Sea partially the same data sets (MWTB boxcore data) have been used for the National Benthos Indicator development for the derivation of the internal reference. For the LPI, trends have been determined for large units (coastal and offshore) for the total biodiversity of benthos and for groups at higher taxonomic levels (including worms and shellfish). The diversity as such is of course also an indicator for the quality of the underwater environment. The plan is that in the near future at least for the offshore area (NCP from the -20m NAP line), analyzes for groupings will take place on the basis of species characterizations (verbal communication Martin Poot). It will then be interesting to compare LPI and BISI results where the LPI might be useful for testing the internal reference. With regard to the development of the National Benthos Indicator, consultation of Statistics Netherlands (Arco van Strien and Martin Poot) has been particularly useful because of their reflection on the methodology under development. Aspects that they have presented, among other things, and to which the method has been adapted, concern working with a geometric mean (log-transforming of the data and back-transforming the result) instead of an arithmetic mean, and evenly limiting (cut-off at both factor 100 exceedance and undercutting) of the reference value for individual species (IIS values) of the BISI index. This ensures that the index is equally sensitive to quality improvement as to quality deterioration, and that in addition to the fact that the index can deal with missing indicator species, there will also be no undue influence on the assessment of highly abundant species (Buckland et al., 2011; Van Strien et al., 2012).

Tests of concept ideas:

During the development of the National Benthos Indicator frequent consultations took place with the clients and a support team of specialists consisting of policy makers and scientists (see also the acknowledgements). Amongst others there have been two workshops in which (1) a plan of approach including the directions of thought and (2) the methodology as developed at that time were presented and discussed (see Appendix 2). Prior to this, a first draft version of the report was circulated, containing examples of elaboration and application, on which a large number of people involved commented. Partly following from the discussions with clients, the coaching team and the workshop, specific adjustments took place in consultation with Olivier Beauchard and the methodology was presented and discussed with Statistics Netherlands (Arco van Strien and Martin Poot). In addition, there were activities that were not directly part of the project described here, but which did follow from there and which provided a great opportunity to test the methodology and ideas. For example, the methodology in development was presented and discussed during the tri-national Dogger Bank workshop in Hamburg, 8 February 2017 (Fock et al., 2017). In addition, the authors are involved in the elaboration of MSFD factsheets belonging to the Marine Strategy Action Plan (part I) 2018-2020, and the first author has developed the factsheet 'Benthic Indicator Species Index (BISI): D6C3 / 5', which in fact is the first application of the methodology to T0 (results based on the MSFD benthos monitoring of 2015).

3.2 Development of a scalable approach

Combining different methods on scales:

The methodology should be applicable for different areas, EUNIS ecotopes and habitat types.

In principle, a specific internal reference with an area-specific set of indicator types is drawn up for each area to be evaluated. On the one hand because each area has a unique ecotope composition with associated potentially occurring species (and therefore also indicator species). In addition, the sensitivity of various types of disturbances to a certain extent is ecotope-specific (especially where it

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has a direct impact on the soil). On the other hand, the monitoring programme also determines the possibilities to merge (sub)areas for the evaluations. This not only applies to the sampling method used (e.g. boxcore, dredge or video transects that cannot simply be combined, apart from the fact that aspects such as sampled surface, the mesh size of the sieves used, selective selection of species, etcetera can also play a role), but also the design of the monitoring programme.

Stratified, randomly executed and / or fixed-patterned sampling strategies that can vary in sampling density per area cannot simply be combined. To a certain extent, MSFD sampling is geared to the (sub)areas to be evaluated where samples are specifically intended (or not intended) for the evaluation of ASEVs and / or the evaluation of the effectiveness of measures. When it comes to the evaluation of EUNIS ecotopes and HD habitat types, samples from different areas often need to be combined. This often requires an intermediate step in which developments within and outside ASEVs (possibly first based on one of the sampling techniques) have to be compared. Since basically work is done with an established sampling programme, when clear differences between sub-areas can be observed even where the sampling intensity differs, it is still possible to combine these sub-areas in a joint analysis. However, the cause of any observed changes needs to be checked (possibly in one of the merged subareas).

From ASEVs to ecotopes and habitats at national level:

Another aspect of the evaluation of larger units, such as the ecotope 'deep sandy substrate' that contains large parts of several ASEVs and other areas, is that a unique reference with indicator species should be drawn up.

Since ASEVs are basically selected to be representative of larger units and are often dominated by a particular type of ecotope (at EUNIS 3 level: EUNIS, 2016) (Wijnhoven et al., 2013), the reference for certain areas with specific ecological values are usually (partly) taken over, and / or combined.

It always has been checked whether indicator species are specific to the relevant ecotope (or can be linked to another ecotope that may also be present in an ASEV whose reference is involved) on the basis of the habitat description in World Register of Marine Species (WoRMS, 2017).

For Habitat Directive habitat types (e.g. H1110b which include 3 ASEVs), the ASEVs references are averaged in relation to the sampling effort per area. Species specific to a particular part of an area (e.g. only present in the northern part and thus not a good indicator for the whole area) might be excluded.

The method is therefore scalable to the extent that it can deal with changes in sampling intensity and is applicable in areas of different sizes, but that a specific reference must often be drawn up, which must therefore be substantiated. It is therefore not possible to apply the current methodology directly to any randomly selected area with variable size. However, the basic method can be set up with some preliminary work for use anywhere.

3.3 Selection of indicator species

'Smart' species as the basis:

The selection of indicator species was initially carried out during the study in 2013, based on an extensive literature review by the NIOZ and expert consultation (workshop and written submission and substantiation of indicator types). In fact, species for which a specific sensitivity to certain pressures is assumed have been selected based on morphological and ecological properties (also known as 'biological traits', among others Bolam et al. (2014), Beauchard et al. (2017)). However, species are classified as much as possible on basis of knowledge from scientific literature (experiments and field studies) and after consultation of experts with field knowledge and a search for confirmation of indicator value on the basis of traits.

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Specific 'biological traits' that have played a role in the classification of potential indicator species are the potential size and age of species and the reproductive strategy (see Table 3, categories C, D and E).

Biological properties such as the creation of tertiary structures and bioturbation or bioirrigation activity can respectively be translated in the interest of the species for the habitat diversity (Table 3, category H) and for the biological activation of the soil (Table 3, category I).

For determining the susceptibility to soil disturbance (Table 3, Category A) the usual depth of the species in the sediment was taken into account, but only used as the only criterion when specific information for the species was missing in the literature but confirmed for closely related species with the same position in the sediment (e.g. a species from the same genus). Selection and assessment are described in detail in Wijnhoven et al. (2013). A term introduced in Wijnhoven et al (2013) is that of 'smart species', which indicate indicator species (specific indication value for the presence of a specific pressure, or representative of a certain effect on the functioning of the system), of which population fluctuations also potentially detectable with a realistic monitoring effort. A realistic monitoring effort means that a limited number of samples with the current methods of the MSFD monitoring programme are sufficient to be able to observe significant population changes (indicative 50% change in abundance or probability of being detected) between two measurement moments, which has been tested on the basis of observed recent observations (average \pm variance). The current MSFD monitoring programme is geared to detecting the occurrence and changes for 'smart species' and typical species.

Inclusion of typical species:

The typical species are species that under natural conditions do not show large population fluctuations at stable conditions, with indication of good abiotic status (Ca), species that under natural conditions do not show large population fluctuations with indication of good biotic structure (Cb), species that under natural conditions do not show large population fluctuations with indication of good abiotic condition and good biotic structure (Cab), characteristic species (K) and exclusive species (E), as identified for the Habitats Directive habitat types. After the study by Wijnhoven et al. (2013), the list of typical species has been extended/actualized, so that the National Benthos Indicator now uses the most recent lists of typical species for H1110 and H1170 (Min EZ, 2014a, b).

Expanding the selection of indicator species for increased reliability:

To reduce the chance that evaluations could accidentally show changes that are not directly related to changes in pressure factors, it is desirable that multiple indicator species are combined in the evaluations. This applies not only to the general quality assessment based on the total set of indicator species per area to be evaluated, but also to specific evaluations to detect causes and consequences of quality changes. Since the testing with regard to the suitability of indicator species ('smart species') or in other words the calculated number of samples needed to detect change has been fairly conservative (see below 'tuning sensitivity'), it is permissible to include species that initially were not included in the evaluations of 2013, to be selected as indicator species. Species that had an indicator score of 1 or more in Wijnhoven et al. (2013) were added if they proved to be well detectable with the monitoring techniques in use. In addition, species with a good indication value for several specific evaluations that consistently appeared to be numerous in certain areas in the past (but are largely lacking now) have been added (based on Bergman & Van Santbrink (1998), De Bruyne et al. (2013), Lavaleye et al. (2000) and Van Moorsel (2002)) because this concerns potential numerous and characteristic species.

Specific evaluations:

The assumption is that a reliable evaluation needs be done on the basis of at least 5 indicator species. Results from specific evaluations based on fewer species are not taken into consideration. The indicator species composition per area to be evaluated therefore consists of a mix of sensitive species, characteristic (area-specific) species and well-detectable species with indication value.

Table 3. Overview of the different evaluations (general + specific evaluations), and the number of categories that have been distinguished to classify types. In addition to the fact that the mentioned indicator values are used in the evaluation based on BISI, they also play a role in the selection of the indicator species.

Code	Causes and effects of quality changes (specific assessments)	Description	Indicator value (IV)
	General quality	Each species has by definition an indicator value of 1 (otherwise the species is not seen as a suitable indicator species).	1 (by definition)
A.	Seafloor disturbance	Combined indicator value for a complex of disturbances (different causes, intensity, frequency).	3 levels (0, 0.5, 1)
B.	Ecological disturbance	Combined indicator value for effect of eutrophication, pollution and toxic substances, oxygen poor conditions and temperature increase.	3 levels (0, 0.5, 1)
C.	Intensity of seafloor-disturbing fisheries	Indicator value based on the size of species (large species are damaged more quickly and / or fished away at low seabed fishing intensity, while populations of smaller species are only significantly influenced at very high intensity).	4 levels (0.25, 0.5, 0.75, 1)
D.	Frequency of seafloor-disturbing fisheries	Indicator value based on the age of species (populations of older species can already be influenced at low soil disturbing fishing frequencies, while populations of short-lived species are expected to be affected only with frequent occurrences of seafloor disturbing fisheries).	10 levels (age divided by 10, IV of 1 by age >10)
E.	Recovery	Indicator value based on the frequent occurrence of recruitment (such types are good indicators for the first phase of recovery).	4 levels (0, 0.1, 0.5, 1)
F.	Characteristic species	Species are almost exclusive or much more numerous in the area to be evaluated than elsewhere in the Dutch North Sea.	3 levels (0, 0.5, 1)
G.	Foodweb structure	Species are important food sources for higher trophic levels (fish, birds and marine mammals).	3 levels (0, 0.5, 1)
H.	Habitat diversity	Species that create permanent tertiary structures, which form a specific niche for various other species.	3 levels (0, 0.5, 1)
I.	Biological activation of the seafloor (top layer)	Species responsible for bioturbation and bioirrigation that play an important role in the ecological functioning of the system (including recycling of nutrients, degradation of contaminants, making the sediment suitable for other species).	2 levels (0, 1)
J.	Typical species of the Habitats Directive	Species designated as typical species for habitat types of the Habitats Directive are assumed to be of importance for biotic and / or abiotic processes and / or characteristic or exclusive for a specific habitat type (H1110 & H1170; Min EZ, 2014a, b).	2 levels (0, 1)

Since no selection has taken place aimed at a specific indication value (and therefore no species indicative of a particular disturbance have been selected earlier than species indicative of another disturbance), the assumption is that the indicator species selection gives a representative description of the general quality state. By zooming in on selections with a specific indication value, insight can be

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gained into the possible causes and potential effects of observed quality changes. These are called the specific evaluations. The specific evaluations are largely in line with the categories selected in 2013 on which potentially 'smart types' have been assessed. Table 3 provides an overview of the specific evaluations and the number of levels that are distinguished to characterize species.

3.4 Tuning sensitivity and determination T0

Working according to comparison with T0:

The T0 (BISI calculation based on 2015 monitoring data) is the first time, and to date the only data set available, in which the monitoring was carried out according to the MSFD monitoring program. The T0 monitoring is therefore representative of what can be expected in the future in terms of numbers and type (different sampling techniques) samples per areas to be evaluated and the spread in the data per area. On the other hand, the 2015 monitoring plays a special role in the methodology as it functions as the T0 as a reference point for future evaluations. Although the implementation of a T0 evaluation is not a specific part of the current assignment, and in this report the 2015 assessment is not specifically presented and discussed (see Chapter 5.5 Recommendations), the results of this monitoring are part of the methodology because in principle every future monitoring moment will be compared with the T0.

Choice of realistic reference instead of pristine reference:

In principle, one could evaluate against a pristine / original reference as far as we are able to reconstruct it in sufficient detail. However, it is expected that firstly such a reference is not a realistic goal, since the habitat constitution of the underwater environment and especially the seafloor has been altered by man to the extent that it is unsuitable for various original species to settle there or to achieve the reference-abundance. In addition, the transition of habitat constitution is largely irreversible, and will largely not recover through natural development after the removal of the dominant pressures. At most, something may be achieved on a small scale by means of restoration. Secondly, we now also have to deal with other species that could potentially have the potential to colonize environments with reduced pressures or build up populations (the species pool present is not comparable to an original reference). Although it is very useful to reconstruct an original reference from the point of view of the perception of what impact the human being has on the underwater environment, quality improvements will most probably not be visible as a development in that direction, because the initial situation (habitats and species) differs greatly. Thirdly, with regard to the detectability of changes, it is also not desirable to work with a reference that is far away from the current situation where changes may not be noticed because they are minimal compared to the reference.

As an example of the original reference of which it is highly unlikely that it will recover in the short to medium-long term through natural development, may serve the extensive *Ostrea edulis* (European flat oyster) reefs that have occurred at the Central Oystergrounds until the end of the 19th / beginning of the 20th century. The literature refers to a contiguous area of more than 25,000 km² at that time (Olsen, 1883, Van Duren et al., 2016). Flat oysters require, among others, attachment options that are largely missing at the moment and will most likely not occur naturally in the foreseeable future. The flat oyster is one of the original reference species, but with the reefs a whole community was obviously associated (habitat type biogenic reef destroyed by fishing) that is now lacking.

Considering the disadvantages of an original reference, it was decided to work with a realistic reference, compiled on the basis of relatively recent observations or estimates based on recent developments. The assumption here is that maximum observed abundances and / or distribution patterns over the past decades give a picture of what is achievable for indicator species. We have based the realistic references on observations of the past 30 years (with the exception of a few older data for the Vlakte van de Raan, because more recent data were available on a limited basis). It must therefore be said that the data availability also plays an important role in this.

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Recent historical data for derivation of the reference:

Datasets and / or data presented in reports that were available and used for the derivation of the realistic reference (also referred to as 'internal reference') are:

BIOMON / MWTL North Sea data from boxcore sampling in the period 1991-2012 (IHM, 2017).

WOT shellfish survey data of predominantly dredge samples (supplemented with Van Veen and Suction dredge samples) for the period 2004-2014 (IHM, 2017).

Dredge data for the period 2007-2010 from a number of NIOZ projects made available for the study by Wijnhoven et al. (2013) and presented in distribution maps in Witbaard et al. (2013).

Dredge and supplementary beam trawl data from the BEON project for the years 1996-1997 as presented in Bergman & Van Santbrink (1998).

Video recordings of the Cleaver Bank made in 2011-2012 by the NIOZ made available for the study of Wijnhoven et al. (2013).

Hamon grab, video recordings, diving observations, beam trawling and dredge data from the Cleaver Bank from 2002 as presented in Van Moorsel (2003).

ICES North Sea Benthos Survey data from 1986 with boxcore and Van Veen grab partly presented in Duineveld et al. (1991) and Craeymeersch et al. (1997).

Dredge and boxcore data from Voordelta and the Vlakte van de Raan from the period 1984-1988 of the BOVO project as presented in Seip & Brand (1987) and Wijnhoven et al. (2006).

Dredge data of the Raan Plain from the period 1962-1966 taken by the DIHO for the 'Bodemhappen Open water project'; data presented in Wolff (1973) and Wijnhoven et al. (2006).

An additional dredge sample from 1990 of the Vlakte van de Raan, taken as part of the international MMP (Monitoring Master Plan) programme under the banner of ICES and OSPAR.

Details regarding sampling methods are given in the protocol in Annex 1.

Derivation of realistic reference:

The realistic reference consists of observed maximum abundance or distribution observations, possibly increased if there are reasonable grounds for believing that maximum observations will soon be exceeded. In doing so, it always has been considered whether data are representative of the area to be assessed. In a number of cases references from similar areas have been obtained. For example, if the historical data availability was good just outside the area to be assessed in a similar ecotope, or an ASEV is considered to be representative of an ecotope or habitat type. In several cases, several areas are representative of parts of the area to be evaluated. In these cases, the weighted average of these areas has been used for the derivation of the reference.

Observations of maximum abundance are frequently increased for the internal reference with the standard deviation observed in the data, or doubled based on expert judgement. This concerns indicator species for which the historical data show a recent increase in occurrence (observations in 2015 already approximate the maximum observed value and / or the maximum value is of relatively recent date). It may also concern species that have shown a higher abundance with another (sub-optimal) sampling technique in periods when data are not available with the (optimal) allocated sampling technique. For example consistently higher densities observed in the past on the basis of boxcore monitoring, when recently the species is hardly found with both boxcore and dredge and should preferably be sampled (on the basis of the size) with the dredge. In a number of cases, indicator species have not been observed in the area in the past, but we do expect them when the dominant pressures on the benthic communities decrease significantly. For those species, the frequent occurrence in one or two of the samples (depending on the current sampling effort) is taken as a reference. In Chapter 4.2.3 'Internal reference' a diagram (Figure 3) is shown with an overview of

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which derivation methodologies are applied. In the Assessment Tool (Appendix 2) the exact derivation of the reference is shown per indicator species per area to be evaluated.

A robust method:

It is inherent in working with a reference that there are some uncertainties. It cannot be ruled out that it will appear that the reference of certain species should ideally be increased (or reduced, although the probability seems to be less, since the reference is based on observations). The expectation is, however, that such uncertainties will have little effect on the assessments, since a range of species will be taken into account in the assessment, and the assessment will not run up against the boundaries because indicator types can exceed only up to 100 times the reference. The current internal reference is expected to be used without any problem for the coming years. If necessary, the internal reference can be optimized on the basis of the first assessments (after a few monitoring cycles) and / or findings from applications in other areas. The method is expected to be robust enough that this will not result in a substantially different assessment. This has also been demonstrated in the first calculations with the data from 2015. Assessments based on two sampling methods combined as intended (boxcore and dredge) give comparable results as assessments based only on one or the other method.

An adaptation of the reference requires that past assessments must be repeated with the new reference (this requires a relatively minor effort). The method is also delivered in Excel format (BISI v290517.xlsx) with pre-programmed arithmetic fields, so that an adjustment of a reference value immediately yields the new result.

Detecting quality changes over time is the strength of the methodology:

As indicated, according to the BISI, the National Benthos Indicator for the North Sea is specifically intended for assessing changes in the quality state and identifying the relative importance of specific potential causes and effects. This concerns the assessment of the quality change at a certain point in time with respect to the T0. Ideally, quality changes are evaluated against a T0 of before measures have been taken so that changes can also be expected. A postponement of these measures or a 'T0' at the moment that measures have already been taken is however not a problem for the method itself. In the latter case it is less certain what underlies the observed quality changes as this can also be a 'natural' development in the quality situation, or an autonomous trend. A special evaluation concerns the assessment of areas with specific measures compared to areas without those measures, on which the monitoring program is tuned so that a BACI (Before-After-Control-Impact) design can be tested.

For a quality assessment at just one moment the influence of the reference will be more profound, because this is not a relative assessment of the development. This also holds for the T0, the moment at which only one complete monitoring according to the MSFD monitoring programme has been carried out, and internal references for certain areas have not yet been tested.

It is not said that such an assessment is impossible: Mainly in relation to the relative importance of causes and effects of the perceived quality situation, the reliability is expected to be reasonable. The uncertainty is mainly caused by the comparison of the quality levels between different areas (with different indicator types, reference values, monitoring efforts, etcetera), which means stacking of uncertainties.

Statistical power of the tests:

Power analyzes were performed to calculate the required number of samples needed to be able to demonstrate at least a 50% change in populations between two moments in time (Wijnhoven et al., 2013). Working with some uncertainties, that in 2013 concerned, amongst others, the availability of data; it has in any case been a standard procedure for the various areas to be compared. It can be said that the calculation has been quite conservative because:

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- Analyzes are based on independent t-tests. This is indeed usually the case for comparison with the internal reference. In cases where quality changes are compared to the T0, these are however often paired comparisons. In cases of assessments of the effectiveness of measures, these are generally according to a BACI design. Both provide a greater power of the tests.
- Power analyzes are often based on a limited dataset (relatively large variance due to the low number of samples). With the current MSFD monitoring programme, the number of available samples is usually larger than used for the power analyzes, so that the actual power is expected to be larger.

It is convenient that there is still some room in the detectability of differences, because 50% difference can be quite substantial. In addition, the set of indicator species has been expanded substantially, but also concerns species that are currently present in very low densities (or even lacking), for which detection of differences can be more difficult. On the other hand, the chance of accidentally observing changes that are not directly attributable to changes in pressures is smaller when more indicator species are included in the analyzes. In principle it is also possible to determine the power of the tests for the current designs (although it needs some effort). It is however less important to know the exact power of the tests since the BISI takes the observed variances into account in the assessments. Moreover, the first assessments and calculations based on the T0 have shown that frequently significant results can be found, taking into account the quality changes to be expected.

3.5 *Elaboration in clear protocols*

Various options and formats have been reviewed (for the presentation of a methodology in a protocol). We have opted for the current protocol (Annex 1) that has been set up for the presentation of indicators within the ICES working group WGBIODIV. The template fits in well with the fact sheets and protocols in use within Helcom, OSPAR and DEVOTES, but is still under development. In any case, we initially opted for an English protocol in view of the current interests to apply the methodology internationally, such as in tri-national comparative work around the Dogger Bank (Fock et al., 2017) and the plans for the indicator to be introduced within OSPAR. At the moment we have not yet chosen to cast the indicator in the OSPAR format because the emphasis at the moment is on testing the indicator under various circumstances. (For extensive testing we preferably have data from a second monitoring event according to the standard monitoring programme available, or have the opportunity of application of a tuned BISI to areas with several consistent years of monitoring). At this stage, the focus is still on the applicability of the indicator, not on the format.

The methodology, on the basis of the protocol (Annex 1), should in principle be feasible for the (sub)areas, ecotopes and habitats of the Dutch North Sea for which the methodology has been developed in the first place. Therefore the protocol is delivered combined with an Excel file in which the various evaluations have been worked out (BISI v290517.xlsx). By using pre-programmed calculations, it is possible to enter the averages \pm standard deviations for the indicator types as observations (Oi) for each monitoring year, after which the assessment (general quality assessment and specific assessments will be calculated automatically. These should then only be compared with (or tested against) the T0 or other evaluations (if several evaluations have already been carried out).

4 Methodology

4.1 *Benthic Indicator Species Index (BISI)*

The calculation:

The occurrence (spatial detection probability) and / or the densities (n / m^2) of a region-, ecotope- and / or habitat-specific selection of indicator species for a particular evaluation moment is / are compared with a composite (internal) reference for the same selection of indicator species. The method consists of calculating a (geometric) average of the weighted log-transformed observation-reference ratios that

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is back-transformed, whereby observed variances in the observation dataset are included in the statistical testing of potential differences.

An evaluation of the quality development of a certain area, or the relative quality development of areas with respect to each other, always consists of a general quality assessment and some specific quality assessments. A general quality assessment shows whether there are developments, or what the quality situation is, based on a set of area-specific indicator species that are all equally important in the assessment. The specific quality assessments are based on subsets of indicator species for which an assessment-specific indication value (a certain weight in the assessment) is allocated. The specific assessments are intended to give an explanation of the possible causes and consequences of the observed quality situation or changes in the quality situation.

As a formula, the calculation of the Benthic Indicator Species Index (BISI) is as follows:

$$BISI = \exp((1/S) \sum (IV_i) \log(O_i/R_i)),$$

S = the number of indicator species in the evaluation of the relevant area,

IV_i = the species-specific indicator value (value determined per specific assessment),

O_i = observed abundance of species i at the time to be evaluated,

R_i = reference abundance for species i (internal reference).

The methodology is described in more detail in Annex 1, on basis whereof an assessment in principle should be able to be carried, given the examples and given the references with indicator values per species for each of the areas to be evaluated. For the application of BISI the provided Excel-file (BISI v290517.xlsx) can be used, in which the calculations for the T0 have been carried out in preprogrammed matrices. Only the measured values have to be replaced for the next evaluation moment, after which the BISI assessment with respect to the internal reference is automatically displayed. For details see: '5. Application'.

4.2 Specifications

BISI consists of a composite realistic reference, based on maximum observations of the past 30 years, possibly doubled or increased (or doubled and increased) with the observed standard deviation for specified areas to be evaluated or derived from similar areas. This makes the method applicable to established areas based on a fixed monitoring program.

4.2.1 Areas (pre)included in BISI

The elaboration for areas of the NCP:

The National Benthos Indicator has been specifically developed for the 8 areas with special ecological values (ASEVs), 6 ecotopes (according to the EUNIS classification at level 3) that describe the Dutch Continental Shelf (NCP) surface area, and 3 HD habitat types (i.e. H1170, H1110b and H1110c). Within the ASEVs, evaluations of the effectiveness of fishery measures taken can be made, whereby developments in sub-areas are compared with each other. In principle, the same internal reference is used for this, and it is only another dataset (specifically assigned dataset) that is used for this purpose. In principle, evaluation of the ASEVs takes place on the basis of data obtained by means of boxcore sampling and dredge sampling, with a specification of the used method per species. In the case of the evaluation of the effectiveness of fishing measures a specific monitoring design is maintained with a comparable sampling within and outside 'closed' areas to increase the power of statistical testing. As a result of a cost-based trade-off, only dredge data are available to test effectiveness of fishing measures. For a number of 'boxcore-species' the evaluation of management is then carried out on the basis of dredge data, for which a dredge-specific reference has been established, in order to allow an optimal number of indicator species to be included in the assessment. An exception is the ASEV Cleaver Bank where evaluation of the quality situation takes

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place on the basis of Hamon-grab and video tracks. Both techniques are also used for the evaluation of the effectiveness of measures. Annex 1 shows detailed methodology (different references) for all areas of the NCP to be evaluated.

Possibilities for applications outside the NCP:

It may be clear that for the evaluation of additional areas, regional references must be drawn up. When setting up new management units (for example closing an extra area for seabed fishing), the evaluation of the area in which it falls may be largely taken over if evaluation is desired. However, for the effectiveness of the evaluation it can be of benefit to check for a number of species whether a different sampling technique can be used to extend the reference list. Of course, specific sampling is also required for the relevant new management unit.

Shifting boundaries:

Whereas the boundaries of ASEVs and sub-areas with specific fishing measures are in principle fixed, the location of EUNIS ecotopes and HD habitat types may vary. In practice, however, the shifts most probably will not be very large. In principle the methodology is developed to evaluate the quality status of fixed areas (and associated sampling locations). With regard to EUNIS ecotopes, therefore, the proposal is for the time being to keep the current maps, and when a new map is available, at least evaluate the shifts (in surface area) and possible consequences for the assessment. For the HD habitat types for which also the observed surface is part of the HD assessment, new contours can always be adopted, but quality change must always be seen in relation to surface change.

With regard to the EUNIS ecotopes and habitat types to be evaluated, shifts can be expected in terms of location and surface in the future. Although not too far-reaching, it may mean that with the introduction of a new ecotope map and / or boundary of a certain habitat, other sample locations will fall within or outside the area; in the past the opposite. The consideration then is whether one is interested in the change in state compared to the T0 whereby the possible occurrence of species belonging to another ecotope type may mean a reduction in quality or that the new ecotope will be maintained, which may be of lower quality because the community encountered previously consisted mainly of pioneer species. In practice, the evaluations will be based on a large number of samples and these transitional samples will be only a small percentage of the total. With regard to the EUNIS ecotopes, the proposal is to start from the currently used map (v2016), and when an update is available to analyze the surface changes and the consequences for the evaluation with regard to the sampling locations that then will belong to another ecotope. A comparison of the quality situation for ecotopes with two different maps for the same year can then provide insight into whether there are consequences of a switch. With regard to HD habitat types, it is obvious that shifts will be observed every 6 years. Quality changes should always be seen in relation to surface developments (something that is already an important part of the HD assessment).

4.2.2 Sampling methods

Choice of sampling method for species:

There are several species of benthos that can be sampled with different sampling gears. Often, one of the methods is more ideal and / or more reliable because either the chance of coincidental observation increases or predominantly juveniles are being caught. On the other hand the mesh size can be the cause that no representative image of the occurrence of a species will be obtained. Often the sampling method to be used for the analyses for a species is also fixed because the species is not detected or distinguished by other methods. In those cases where several methods are potentially useful, it may be possible to use a suboptimal method (which does not say that data is unreliable) in the evaluation in case there is only one method available for the type of evaluation. For example, there are several species for which the observations in the evaluations of ASEVs are based on boxcore data, while in the evaluation of the effectiveness of fishing measures dredge data for these species are used. In those cases, reference values also differ for that species because they are

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specific per sampling method. Annex 1 shows elaborated methodology with the indication of the sampling data used per indicator species per area to be evaluated.

Dealing with differences in sampled surface:

One aspect that potentially needs attention is the sampled area both per sample location and per area to be evaluated. Ideally, a standard methodology with standard sample surface is used during the monitoring in connection with the data on the basis of which the reference is derived. The current benthos monitoring programme includes sampling with standard boxcorers (0.078 m²) and standard Hamon grabs (0.09 m², only applied to the Cleaver Bank). This makes the samples taken with the same method by definition comparable, assuming that the entire sample is always sorted out. In practice, subsampling for certain species will take place and have taken place. Since this is only permitted under strict conditions and thus standardized, we can assume that this does not substantially influence the estimate of the densities per species, and that all species present are detected. For derivation of the reference, frequently data from samples with other surface sizes were used.

Since the historical data have been used to derive the maximum potential for indicator species, the sampling surface used in the past is not very important assuming that the monitoring gives a representative image of the area in question. At most there is a risk of under-sampling, which means that relatively rare species, or species that are naturally present in low densities, are missed. The probability of this is, of course, limited if data are available for various reference years for different periods (e.g. 80s, 90s and the beginning of this century). The (historical) data availability plays a role in the expected representativeness of the maximum potential that is observed. It is then up to the expert (author of the methodology for a specific area) to determine whether the maximum potential in the observation years was to be expected, or whether the sampling intensity had always been sufficient. Subsequently, based on the estimate, it is determined what a real reference is that can be observed in the substantial reduction of the dominant pressures over the medium term. In all, but especially in that last aspect, there are uncertainties. We have chosen to make the best possible estimate of a realistic reference per species, which means that we base ourselves partly on historical observations, but how to deal with it has been an expert assessment, following fixed standardized options (see 4.2.3 Internal reference). If the method may need to be used in the future for other geographical areas with a completely different species composition and results must be compared with each other, further standardization of the derivation of the internal reference will be desirable.

It needs to be realized that a certain degree of variation in sampling occurs that is not random, but for which it is questionable whether compensation for this in the method provides any improvement. For example sediment sampling depths of sampling devices depend on the type of substrate and determine the species that might be missed. Considering the possible sources of variation, it can be assumed that a boxcore sample of 0.078 m² and a Hamon size of 0.09 m² (for which the entire surface is not as deep) are comparable to a certain extent.

As far as the dredges and video tracks are concerned, we know that the inventoried surfaces in the past and also at present still strongly differ, with the data generally being converted to a standard size. In fact, one can only state that a sample should be representative of the sampled area, and that at most a minimum area that needs to be inventoried to prevent species present in low densities from being erroneously missed. But what is often not fully controlled is whether a relatively homogeneous substrate is sampled or that there are other habitat elements with different species or densities. For this purpose, it is desirable to make relatively short hauls (if one can already collect or analyze the material). Also, the sediment penetration depth of corers, again partly dependent on the substrate, determines the chance of catching species. Working with derived maximum potential observations from the recent past and at least as many as possible standardized lengths of hauls from 2018 onwards, it is expected that conversions to standard surfaces (and consequently the elimination of species with low densities if larger surfaces have been sampled) do not yield greater accuracy.

Established benthos monitoring program prevents extra variation:

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The fact that in the future a fixed monitoring programme will be used means that certain sources of variation are more or less the same each monitoring year, so that the comparability of campaigns is large. For example, variation in sediment sampling depth related to substrate type will occur to the same extent at the same locations. This even means that the fact that dredge samples in the coastal zone are locally replaced by Van Veen or suction corer samples is not a problem with regard to the evaluations, since they are being applied standardized at the same locations.

The monitoring effort per area to be evaluated is geared to the 'natural' variation for potential indicator species as determined in an earlier phase (Wijnhoven et al., 2013). If the realized monitoring effort will deviate only a little (this is the expectation in view of the established monitoring programme), it is expected that detecting differences in the occurrence of indicator species are real differences (and not the result of differences in the total surface area sampled).

4.2.3 Internal reference

Internal reference

The internal reference is a composite realistic reference with respect to the abundance of benthic indicator species (meaning that by largely eliminating the prevailing pressures the described state can be achieved naturally from the current habitat composition and present species pools) that are being used in the BISI methodology to compare the situation at a time to be evaluated.

A reference value is used in the BISI to reflect the desired condition of the benthos. It has been decided not to work with an original / pristine reference, because it is unrealistic that by natural development after minimizing or eliminating the pressures on the benthic communities such a reference state will be achieved. The seabed environment has been intensively influenced by humans for more than a hundred years, so that the habitat composition is not comparable with the original reference situation. In addition, there is a completely different species spectrum of potentially recurring and / or settling species in case of quality improvement. Therefore, a realistic reference is used, which describes the communities as well as possible in case the dominant pressures are removed, based on the current situation. By using recent historical (mainly past 30 years) abundance and dispersion observations (see 3.5 'Tuning sensitivity and determining T0' for the datasets used) for compiling the reference, the reality of the reference used is guaranteed to a certain extent.

Derivation of reference values:

The derivation of the reference value per indicator species per area to be evaluated has been standardized as much as possible (Fig. 3). Depending on recent developments of indicator species, the current level of the abundance, possible observations from other (suboptimal) sampling techniques, an estimate of the representativeness of the sampling programme at the time of the historical observations and the observed variance of the data, the maximum observed values are being used as a reference. Those maximum observations are possibly doubled or increased with the observed standard deviation (or both). In a number of cases the suboptimal observation has also been halved, or the occurrence in one or two of the samples has been used as a reference. It has been decided not to fully define the derivation method for the reference values, but to allow some (well-founded) choices. With this, the sense of reality has been preferred above the total / strict standardization of the derivation method, to allow for some adjustments based on expert assessment in the derivation process.

The choice of using reference values in the evaluation:

The advantage of an evaluation with respect to a reference is that observations can be assessed in perspective, although the establishment of a reference necessarily entails uncertainties. A doubling of the abundance of one indicator species may be a much more important indication than for another

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species, depending on whether these developments will approach the reference value, or whether for example a doubling still means that the species is far removed from the reference value.

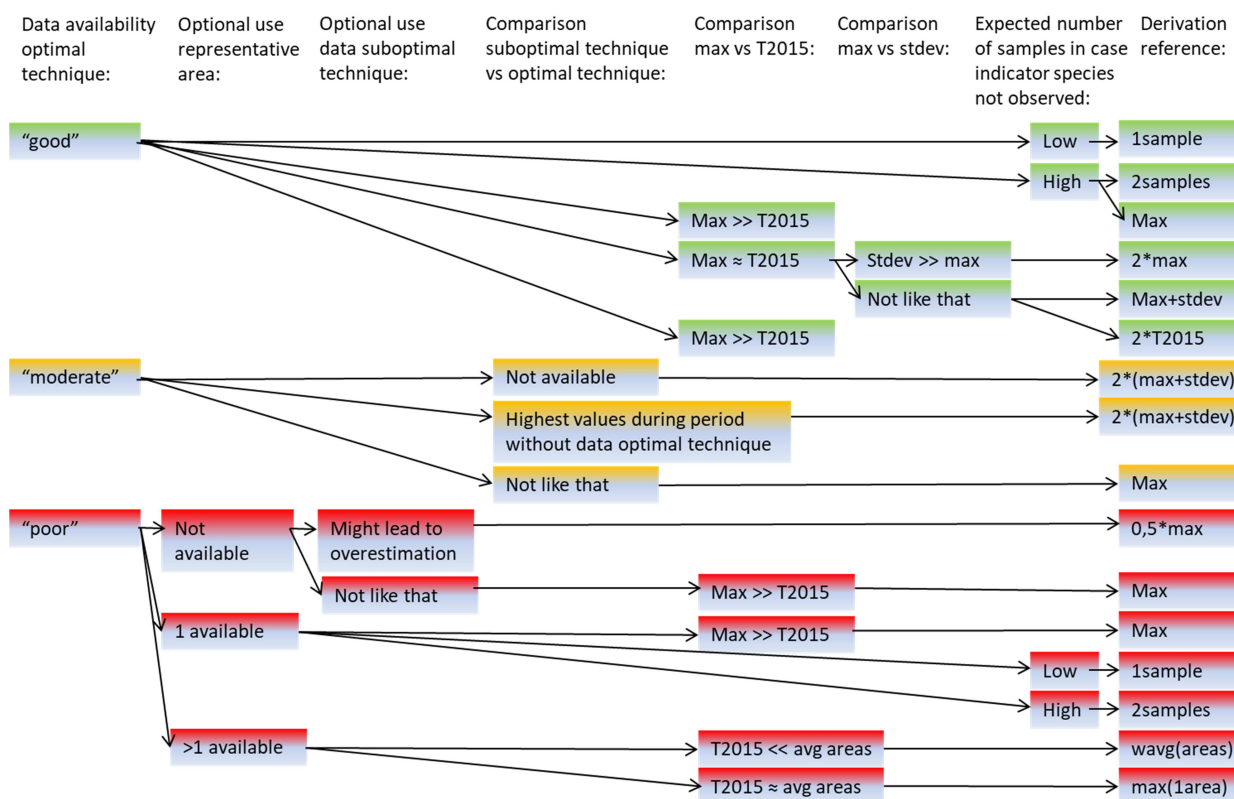


Figure 3. Overview of selection scheme for internal reference. For the exact reference values and the derivation method per indicator species per area to be evaluated, see the BISI Assessment Tool (Appendix 2). Max = maximum annual mean as observed in recent historical observations; stdev = standard deviation; T2015 = the annual mean value as observed in 2015; 1sample = a density comparable to the occurrence of the species in one sample; wavg = weighted average of different areas based on the number of samples taken; >> = much bigger; << = much smaller; ≈ = comparable values.

If the reference is exceeded in a positive way and the data is only cut off at 100 times overrun (and, moreover, undercutting), the exact reference value becomes less important. This makes the method ideal for assessing quality changes over time or changes between differently managed areas based on a specific sampling design. Although the assessment of the exact quality at one measurement moment or the comparison of areas with different references and sampling efforts is not the strongest point of the methodology, due to the use of a range of indicator types, the method is robust enough to make even reliable statements about the quality status on the basis of one sampling year (such as for the T0). In addition, the identification of causes and consequences of the perceived quality value provides important insights. Annex 1 shows detailed methods (including the composite internal references) for all areas of the NCP to be evaluated.

4.2.4 Data type

Evaluation of different data types possible:

Although in general the evaluation is based on the abundance of indicator species in densities (n / m^2), the method is in principle suitable for using and / or combining different types of observation data.

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In some evaluations use is made of the spatial detection probability for species. This is particularly interesting when the species naturally has an irregular ('patchy') distribution, and can then be locally abundant, or when species have large seasonal fluctuations and can manifest themselves early in the year (monitoring is normally performed in early spring to minimize the effect of seasonal fluctuations). It is important, however, that for the use of chances the samples are needed with a fixed area and no subsampling has taken place (which is in fact a reduction of the sampled area). Because this is only the case in the current MSFD benthos monitoring programme for the boxcorer and Hamon grab, spatial detection probability is now only used for these methods. Of course, the data type of the observations and the reference must match.

Adjustments for the use with respect to Natura 2000:

Current assessments under the Habitats Directive include evaluating the presumed presence of typical species in HD habitat types. The National Benthos Indicator gives the opportunity to substantiate this. Since in the coming years basically a fixed monitoring programme will be used (standard effort), it is possible to assess the presence / absence of, for example, only the typical species (specific assessment J) in the monitoring programme per area (HD habitat type), and can be substantiated if developments take place. In Annex 1 the standard data type is displayed per indicator species per area.

Taking into account sampling efforts for the years to be assessed:

In addition to the type of sampling technique, the sampling design is also important. A skewed distribution of the number of sampling locations (concentrated in a certain area) may give a different outcome of the assessment. In principle, the methodology is based on a random or representative (e.g. according to surface area ratio of the EUNIS ecotopes that describe the area to be assessed) distribution of the samples.

However, due to the existence of an established monitoring programme, the method may also work if the sampling is still concentrated in a specific area. It must then be checked whether the monitoring effort in the years to be evaluated follows the same (oblique) distribution. The monitoring program is in fact a random stratified design that has been defined at a certain moment. The sampling locations for the assessment of ASEVs are expected to be representative (which also makes it less harmful when the data of a random sample is lacking due to certain circumstances). However, this is a stratified design, because the monitoring is focused on ASEVs. As a consequence, when assessing EUNIS ecotopes or HD habitat types, it is necessary to examine whether areas inside and outside ASEVs are of comparable quality. This is entirely the case when certain sampling techniques (for example, the dredge) are only used in ASEVs for specific evaluations. It then needs to be assessed whether the different techniques can all be used for the evaluation, or that selection for a specific technique is required. In general, evaluation cannot be separated from any developments in individual ASEVs. In addition, there are the evaluations of management measures for which sample locations have been designated (following a specific design). Certainly, the samples for comparison (for the assessment of the Dutch North Sea, the samples taken in 'open' areas compared to a specific 'closed' area) may not be representative of all open areas within a certain area with special ecological values (ASEV), but are selected because they are similar to the habitats and / or communities of the closed area (from before the measures). Samples to assess the effectiveness of measures are specifically indicated and cannot be used for other assessments (which should be representative). See '5. Application' for the specific tests that are used to assess the usability of data and the output of the evaluations themselves.

4.2.5 Completeness reference

In fact, the BISI assessment is based on assessing the completeness (intactness) of a reference, where the reference consists of a composite indicator species community. The assessment determines the relative distance of the abundance of a specific indicator species for a specific area to its reference value, after which the outcomes of all included indicator species are averaged. In the

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general quality assessment, all types weigh equally. With regard to (additional) specific assessments to determine the causes and / or effects of perceived quality (changes), each indicator species has a specific indicator value (weight) for the individual results. An indicator value (IV) of 0 means that the relevant species is not taken into account, while an IV of 1 means that the species counts up to a maximum. Individual indicator results (also referred to as IIS values in the calculations = Individual Indicator Species values) are averaged geometrically (the IIS value is the log-transformed ratio of observational value divided by the reference value, multiplied by IV weighting factor), after which the average IIS value again is back-transformed (using inverse log- transformation). This prevents very numerous indicator species from dominating the outcome. In order to ensure that the method can deal with the absence of species (0-value), cut-off takes place when the observation is 100 times smaller than the reference. In case of absence of the species, an observation divided by reference (O_i / R_i) of 0,01 is assumed. In order to make the indicator equally indicative of quality increase and decrease, cut-off also takes place when the reference is exceeded by 100x. For details, formulas and calculation examples see Annex 1.

5 Application

5.1 General application and Excel tool

As indicated earlier, the assessment consists of comparing BISI values (a general quality assessment per area to be evaluated and a number of specific assessments to identify causes and effects of observed quality). In addition to potential differences, observed differences in level between reference - (by definition BISI = 1) and observation moment or between periods or (sub)areas, it is also necessary to test whether any differences are significant, taking into account the observed variance.

The components of a BISI evaluation as shown in the Assessment Tool:

The section below refers to the Excel file 'BISI v260917.xlsx' in which all references for the evaluation (current status) of the NCP are elaborated in separate worksheets. It is always indicated which type of observations should be used for the evaluations. Where references are in principle fixed (or it must be decided that the methodology needs to be adjusted, after which previous assessments should also be assessed with the help of the new reference) the field with observations ($O_i \pm \text{stdev}$), in the spreadsheet holding the data in for the year 2015, can be overwritten with the observations of another evaluation moment.

Indicator species: The worksheets per area to be evaluated (ASEV, EUNIS ecotope or HD habitat type) present the area-specific list of indicator species in the first column. In case several interim evaluations are necessary (for example, comparing inside and outside ASEVs to determine whether data can be used together and / or whether a method only applied within an ASEV can be included in the evaluation of an ecotope type), the same list with indicator types (and all other columns) is repeated a number of rows downwards.

Number of samples: After the column with indicator species follow the columns with the number of expected samples (according to the MSFD benthos monitoring programme) and indication of the method (sampled area). In the calculations, the expected numbers of samples are not used. It is an indication that when the number of samples is substantially lower, it must be checked whether the subset is still representative. What the columns do indicate is which observation method will be used for the relevant indicator type (and this is possibly area- or even evaluation-specific). The number of samples for the observations, in the form of the standard deviation, is however used for statistical analyses (t-tests).

Type of evaluation: Next, the columns follow which indicate which data type will be evaluated. These are mainly 'densities (n / m^2)' and in a number of cases spatial detection probability (as a proportion of samples in which species occur in total).

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Maximum values for averages \pm stdev per historical monitoring programme: Next follow a number of columns with data used to derive the realistic reference. This concerns the maximum observed annual average \pm standard deviation (if relevant and / or representative) for monitoring programmes or parts thereof. They may concern different sampling methods, of which it is always considered whether the data can form a realistic reference. For example, dredge observations are presented for boxcore species and *vice versa*, whereby these values may not been adopted, but could be an indication for whether or not to raise final reference values. (Presented data in these fields are informative).

R_i (the internal reference): This is the composite realistic reference which in principle can also indicate a kind of goal for the quality state, although in many cases a status quo or a significant improvement of the quality state will be the goal (without a specific goal in terms of quality within a set term). Observations will be assessed in relation to the internal reference in BISI. References are specific to applied observation methods, and here too the observation method is indicated.

O_i and O_i / R_i ratio (observations and ratio calculations): Here are always 6 columns, where in the first two the observations are entered as averages with standard deviation. In the spreadsheet, the results of the monitoring in 2015 have been filled in (which will also serve as a T0 that will be compared with observations in the future). If the monitoring data resulting from future campaigns of the indicated method are entered here, the observation reference ratios will automatically roll out (O_i / R_i). If needed, the data in the column 'adjusted O_i / R_i ' are adjusted because cut-off takes place at a 100x larger or smaller than the reference. The standard deviation associated with the ratio can then be found automatically in the next column, adjusted if smaller than 0.01 in order to be able to deal with zero values and because it is assumed that there is always a chance of a minimum of variance (even though it is not directly observed).

IV_i (Indicator values of individual species per specific assessment): Here, according to the attributed indicator values for a number of categories within the first column (General quality), by definition a '1' for the general quality assessment and in the next column the IV for the specific assessments as specified in Table 3 (also on each worksheet under the ratings shortly identified).

(IV_i) $\log(O_i / R_i)$, Individual Indicator species value (IIS value): The same categories of specific assessments can be found here for which the IIS-value is calculated automatically. This is in fact the weighted logarithmic observation-reference ratio. (If the species does not have a specific indicator value, that species is not taken into account and appears in the cell 'not available (na)').

Standard deviation: The same categories of specific assessments can be found here for which the standard deviation pertaining to the IIS-value is automatically calculated. (If the species does not have a specific indication value, that species is not taken into account and appears in the cell 'na').

The results of an assessment by means of the Assessment Tool:

We now have all information for the assessment and now BISI can be calculated. This is done in the rows below the list of indicator species and the provided information. For an ASEV, the procedure is the simplest because only one list of observations needs to be entered.

For the sake of completeness, it is indicated how many indicator species are distinguished per observation method used. A row below the formula for calculating the BISI can be found ($BISI = \exp((1/S) \sum (IV_i) \log(O_i/R_i))$).

When we go to the right, under the list of indication values per species, we find the average IV for the specific assessment, and below the number of indicator species for the specific assessment. We consider 5 indicator species as a minimum for a reliable (specific) evaluation. For some assessments, we do not meet that number (five) of indicator species: those columns are shaded gray and results are not included in the assessment (often this is the case for a specific assessment of little relevance for the area concerned). (In a few cases it might be considered looking for an additional indicator species in the future in order to be able to carry out the relevant assessment).

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BISI values: If we move further to the right, we find in a row below the BISI values (in bold) for the general and the specific assessments. In fact, this is the **red** result where the value indicates how far the quality state is removed from the realistic internal reference. (The corresponding standard deviations follow later in the same row).

Testing observed differences:

Since the absolute difference with the reference which by definition has a BISI value of 1, does not indicate whether it really is a difference, this is (automatically) tested in the rows under the BISI values. This concerns two-sided (quality status may be lower or higher than the reference) independent t-tests (because the reference is often based on a differently designed monitoring than the MSFD benthos monitoring).

Test results: In the 4th row of the statistical results you will find the 'Probability of computed t', the test result that indicates that we are dealing with a significant difference with the internal realistic reference if the value ≤ 0.05 . For the sake of clarity, for the T2015 it is indicated whether differences are very significant (≤ 0.001 ; ***), quite significant (≤ 0.01 ; **), significant (≤ 0.05 ; *) or not significant (> 0.05 ; ns). With regard to a T0 as carried out in 2015, it could be concluded that the quality situation is not yet optimal if a significant difference is found for the general assessment, but that certain potential causes or effects of the quality situation, as indicated by the specific assessments, are less important if not found to be significant.

For the sake of completeness, also synonyms or (possibly incorrect but used) other names for indicator species as mentioned in the reference data are listed under the list of indicator species.

5.2 T0 evaluation of ecotopes and habitats

For the assessment of an ecotope or habitat, it is possible that the assessment cannot be carried out in its entirety at once. For example, it must first be tested whether the initial quality status inside and outside ASEVs is comparable. This can for example be based on the boxcore data. If the situation is comparable, one can indeed for example consider including the dredge data that have only been taken in the ASEVs, in the analysis.

The evaluation of the individual parts inside and outside an ASEV is exactly the same as described earlier for an area with special ecological values as a whole. It is that now only a part of indicator species are included in the assessment (only to follow the species with a boxcore sampling). The BISI calculations remain the same, except that in this case the difference with the reference is not specifically tested, but it is first checked whether there is any difference between the parts inside and outside the ASEV. However, this concerns two-sided independent t-tests. If no significant differences are found, the dredge samples can be included in the analyses (even though they have only been taken within the ASEV). The calculations are again based on the combined boxcore and dredge data and checked against the reference. If you have any doubts, it is possible to only select the boxcore data and test them against the reference.

5.3 T0 assessment of effectiveness measures

In fact, the T0 assessment with regard to the evaluation of management measures is the same as the method described above for 'composite' areas. Here, too, the quality status is determined independently in two areas and compared with each other. It is true that this concerns just dredge samples (with the exception of the Cleaver Bank) and that the samples are specifically designated for this application. It is also possible to consider evaluating closed areas separately or jointly, but this choice has in fact already been made in the worksheets (and actually already in the design of the monitoring program). Test results now show whether the initial situation (often the situation before measures have become effective) is comparable in the areas.

5.4 Tests in the future

After estimation of the T0, it depends on the objectives, implementation of measures and data availability which tests to use. In principle, the future monitoring years will be compared with the T0. Since a fixed monitoring programme is used that is repeated every 3 years, testing for significance can be regarded as a paired t-test (which increases the statistical power). It is expected that from 2028 onwards, probably there will be sufficient data for a trend determination based on the BISI values. However, for a number of areas it is possible that the monitoring will start in 2018 before or at the time of the implementation of measures, with which it may be optional to consider the 2015-2018 data as T0 instead of comparing years with 2015 as T0, and thus to compare the years thereafter (possibly also by merging years) with a double amount of data.

With regard to evaluating the effectiveness of measures, there is clearly a BACI design. Not only the difference between areas with and without measures in a given year is important, but also the starting situation (especially when there were already clear differences in quality at the time of the T0). For the testing of any significant differences, the difference in IIS (Individual Indicator Species) values between the T0 and another moment of observation can then be estimated, so that series with IIS differences per indicator type are obtained. These can be compared by means of a paired t-test (after testing any differences in variance: F-test) for the entire set of indicator species (general quality assessment) or the identified subsets of indicator species (for identification of possible causes and / or consequences of perceived quality differences).

5.5 Recommendations

The strong point of the BISI method is to demonstrate quality changes. This does not mean that the methodology cannot give a quality opinion on the current situation (for example, the situation in 2015 with a monitoring carried out almost entirely for the first time according to the benthos monitoring program). However, the comparison of the quality of different areas is still accompanied by some uncertainties. Verification by applying the methodology for different areas and at different moments in time will also improve the quality assessment at a specific time.

Assessment recent historical situation with BISI:

The quality situation in 2015 is in principle part of the methodology; future evaluations will be compared to the baseline situation. In this way, the situation in 2015 (the T0) is partly incorporated in the current report and the Assessment Tool (Appendix 2). Nevertheless, it is just for this reason that it is advisable to record the quality situation well in figures and tables in a T0 report (in 2015), so that the framework of reference is clear. For a number of areas to be assessed, the situation in 2015 is a real T0 situation because management and protection measures have been implemented around 2015 and any effects are expected in the coming years. It is important, however, that it is clear for each area what the situation is in the process. But also, what the developments in recent years have been if this information can be retrieved. For example, a T0 situation that has been relatively constant for decades might be very different from a quality situation that has been showing a declining or increasing trend for a number of years. A valuable exercise is therefore, when possible (depending on the availability of data), to carry out BISI evaluations for years and / or periods in the past and to present the results as part of the T0. It is necessary for data to be present at all times with the same sampling effort for all sampling methods as part of the assessment. Taking into account the (increasing) uncertainties, any gaps in the data can be filled in which might result in important insights. This will also provide insight into the robustness of the current methodology.

Comparison using an original undisturbed reference:

To put the contemporary results in perspective, it would also be good to sketch the undisturbed original reference for a number of areas (where possible) on the basis of historical data and literature. Although this will often be a situation that will not be realized in a natural way after the removal of dominant pressures, it is important to be able to show that the current situation is the result of

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intensive use of the systems over the centuries. In addition, such an exercise can also make clear which elements and / or areas deserve extra attention and / or protection and can help with targeted recovery measures (e.g. active recovery in the form of local policy of conservation).

Application outside the boundaries of the NCP:

The method is currently specified for the Dutch situation. This does not mean that it would not be applicable for any other area, but before that is possible some aspects still need to be worked out. The most important aspect concerns the internal reference. For application outside the NCP, the selection of indicator species and especially the derivation of reference values must be fully standardized. Although additional problems are not immediately expected when the method is applied in different habitats, based on other species compositions, using other monitoring techniques, in areas where very different pressure factors play a role, etcetera, these are obviously good conditions to test the general applicability of the methodology. It is currently being examined whether the BISI could possibly be upgraded and / or integrated with the existing initiative of the BH1 (Typical Species Composition) as an OSPAR indicator (www.ospar.org).

For application in areas close to the NCP (where the comparable ecotopes are concerned) only minor adaptations of the methodology are required. This mainly concerns harmonization of the reference to the applied monitoring methodology and / or adaptation to any other ecotope surface ratios. In order to test and refine the methodology, application in neighboring areas in England, Germany and Belgium would therefore be very valuable.

Ideally, the methodology is tested in areas with, to a certain extent, known spatial and / or temporal variation in the dominant pressure factor(s). What is lacking in the Dutch application area is a 'reference area' where the dominant pressures are substantially lower, such as areas that have effectively been closed for a long time for all potentially seafloor disturbing activities.

Test and application options within the NCP:

However, suitable test situations are also present in the Dutch situation where, to date, any quality changes can be linked to known variation in pressure factors. The comparison of the quality according to BISI with fishing activities is a good test for the sensitivity of the indicator. For example, there may be good application and testing options for the wind farms that have recently been set up and will be set up, where the designation involves the exclusion of all sorts of activities in the immediate vicinity and, in general, an intensive monitoring is part of the execution of the projects. Among others, the Princess Amaliapark has a monitoring with a good number of dredge samples taken for a number of years since 2003 (personal communication Maarten de Jong). In addition to evaluating (relatively small scale) effects of, among others, closing the areas for fishing activities, BISI can also play an important role in the detection of 'autonomous' developments. This concerns the development of the larger areas in the North Sea in which the wind farms are located, which must be taken into account in evaluations of the effects of wind farms.

Possible adjustments in the future:

In addition to applying the methodology in test situations or other (international) areas, the application for the MSFD evaluations and the evaluation of the effectiveness of management measures may also be reason to refine certain aspects in the future. Although the current elaboration is expected to provide sufficient scope for carrying out the evaluations at the NCP in the coming decades, it may be possible to conclude that the reference for a single individual indicator species needs to be adjusted. It might mainly concern species for which the reference should be increased or progressive insight with regard to the specificity of indicator species for different pressure factors and quality improvements. The method is expected to be robust enough (because assessments are based on the development of a large number of indicator species) so that small adjustments will hardly affect the assessment. However, at the moment of changes in the methodology, prior evaluations also have to be re-calculated in order to keep trend assessments and / or changes in time clear. (This requires a minor effort since making changes to the BISI Assessment Tool immediately produces the new

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results, although it is advisable to save copies of the results of the original and the modified method separately). It is recommended that for each application a section in the report should be devoted to the test of the methodology and report suggestions for improvements in the future.

Applicability regarding HD and Natura 2000:

The method is arranged in such a way that it can be applied for use for Habitat Directive reports and Natura 2000 evaluations. To date, the HD and Natura 2000 have their own system in which the occurrence of typical species plays an important role. The BISI method has specific evaluations of the HD habitat types and specific assessments of the collection of typical species. This makes it possible not only to make a statement about the occurrence of typical species, but also to link these to the sampling effort and thus to evaluate developments in the populations of typical species. With this, quality changes become clear at an earlier stage than when only the number of typical species present is assessed, and changes due to the link to specific pressures and effects and the assessment of different (sub)areas can also be interpreted. Since a BISI calculation (now that the method is available) can be carried out with a relatively small effort, it is obvious to use BISI results at least as additional information or to substantiate reporting within the framework of the HD and Natura 2000.

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Annex 1. Protocol Benthic Indicator Species Index (BISI)

Appendix 1. Reference lists of indicator species for distinguished areas of the NCP to be evaluated

Reference lists of indicator species for each of the areas with special ecological values (ASEVs), ecotopes (EUNIS3) and Habitat Directive habitat types that are (part of) benthos related evaluations of the Dutch North Sea. Indicated are which type of monitoring will be used (and the expected number of samples taken with 3-year intervals starting in 2015); the type of observations used in the evaluation (indicated with '1'; the used reference value (R_i) for evaluation; and the Indicator Value (IV_i) for different causes of and functions subjected to change as indicated by the indicator species. The Indicator Value is shown for the causes and functions: A. Sea floor disturbance, B. Ecological disturbance (combining possible effects of pollutants and toxicants, hypoxia and temperature increases), C. Intensity of sea floor disturbing fisheries (on basis of size of species), D. Frequency of sea floor disturbing fisheries (on basis of age of species), E. Recovery (on basis of frequent recruits), F. Characteristic species, G. Food web structure (importance for higher trophic levels), H. Habitat diversity (species creating permanent structures), I. Biological activation of sea floor top layer (bioturbating and bioirrigating species), and J. Habitat Directive typical species, when relevant. Total numbers of indicator species as part of the general quality assessment and of the specific evaluations are indicated.

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Appendix 1a. Reference list of indicator species for the area with special ecological value Dogger Bank.

	Expected number of samples per year (2015+)		Data type of evaluation		R _i		IV for Pressure											
	Boxcore (0,078 m ²)	Dredge (20m ²)	Hit rate (spatial)	Densities (number/m2)	Boxcorer (0,078 m2); hit rate (per core) or densities (n/m2)	Dredge (20 m2); densities (n/m2)	General quality	A	B	C	D	E	F	G	H	I	J	
Indicator Species																		
Acrocnida brachiata	20			1	160,920		1			0,5	0,4	1	1	1				
Alcyonium digitatum		15		1		0,006	1	1		1	1	0,5	1					
Angulus fabula	20			1	331,013		1	1		0,5	0,3	1						
Aphrodita aculeata		15		1		0,420	1	0,5	0,5	0,75	1	0,1	1					
Arctica islandica		15		1		0,602	1	1	0,5	1	1	0,1						
Astropecten irregularis		15		1		1,195	1	0,5		1	0,5	0,1	0,5					
Bathyporeia elegans	20			1	490,375		1		1	0,25	0,1	1		1		1	1	
Bathyporeia guilliamsoniana	20			1	100,585		1		1	0,25	0,1	1		1		1	1	
Buccinum undatum		15		1		0,184	1	1	1	1	1		1					
Corystes cassivelaunus		15		1		1,595	1	1	1	1	1	1		1		1	1	
Echinocyamus pusillus	20			1	36,863		1		1	0,25	1	1						
Ensis ensis		15		1		5,332	1	1		1	1	0,1	1	1		1	1	
Ensis siliqua		15		1		1,144	1	1		1	1	0,1	0,5	1				
Euspira pulchella	20			1	57,688		1	0,5		0,5	0,3	1		1				
Gari fervensis		15		1		1,184	1	1		0,75	1	0,5	1					
Goniada maculata	20			1	92,950		1				0,1	1		1				
Iphinoe trispinosa	20			1	18,300		1			0,25	0,1	1						
Kurtiella bidentata	20			1	1287,500		1											
Lanice conchilega	20			1	474,040		1	0,5	1	0,5	0,1	1		1	1	1	1	
Liocarcinus holsatus		15		1		0,175	1			1	1	1		1				
Luidia sarsii		15		1		0,040	1						1					
Magelona johnstoni + M. filiformis	20			1	775,638		1			0,25	0,3	1		1		1	1	
Nephtys assimilis	20			1	12,813		1	0,5	1	0,5	0,6	1	1					
Nephtys cirrosa	20			1	43,263		1	0,5	1	0,5	0,5	1						
Nephtys hombergii	20			1	181,420		1	0,5	1	0,5	0,5	1						
Neptunea antiqua		15		1		0,010	1						1					
Ophiothrix fragilis		15		1		0,075	1						1					
Ophiura ophiura		15		1		0,166	1	1	1	0,5	0,5	1		1				
Owenia fusiformis	20			1	73,713		1		1	0,25	0,5	1		1	1	1		
Pagurus bernhardus		15		1		0,728	1	0,5		1	1	1						
Psammechinus miliaris		15		1		0,423	1	0,5	1	0,75	1	1	1					
Sigalion mathildae	20			1	52,888		1	0,5		0,5	0,4	1	1					
Spiophanes bombyx	20			1	3730,780		1			0,25	0,2	1				1	1	
Urothoe poseidonis	20			1	125,000		1			0,25	0,1	1		0,5				
Number of species (S)	18	16					34	19	14	29	30	29	14	14	2	8	29	

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Ophiura texturata* = *Ophiura ophiura*; *Amphiura brachiata* = *Acrocnida brachiata*; *Mysella bidentata* = *Kurtiella bidentata*; *Eupagurus bernhardus* = *Pagurus bernhardus*; *Magelona papillicornis* = *M. johnstoni* + *M. filiformis* (as *M. papillicornis* has never been present in the North Sea).

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Appendix 1b. Reference list of indicator species for the area with special ecological value Cleaver Bank and Habitat Directive habitat type H1170 'Reefs' (as both evaluations are based on the same samples).

ASEV Klaverbank / Natura2000 habitat H1170	Expected number of samples per year (2015+)			Data type of evaluation		R_i	IV for Pressure										IV for Ecological Functioning	Habitat Directive
	Hamon grab (0,09 m ²)	Boxcore (0,078 m ²)	Video tracks (20 m ²)	Hit rate (spatial)	Densities (number/m ²)		General quality	A	B	C	D	E	F	G	H	I		
Indicator Species																		
<i>Arcopagia crassa</i>	17	1			1	0,526	1	1		0,5	1	0,5	1					1
<i>Aequipecten opercularis</i>			16		1		1	1		1	0,6	1	1					1
<i>Alcyonium digitatum</i>			16		1		1	1		1	1	0,5	1					1
<i>Aonides paucibranchiata</i>	17	1			1	962,880	1				0,1	1			1			
<i>Aporrhais pespelicani</i>	17	1		1		0,125	1	1		0,8	0,3	1	1					1
<i>Arctica islandica</i>	17	1		1		0,250	1	1		1	1	0,1						
<i>Buccinum undatum</i>			16		1		1	1	1	1	1	0,1						1
<i>Cerianthus lloydii</i>	17	1			1	88,222	1	1		1	1	0,1				1		
<i>Chone duneri</i>	17	1			1	217,350	1	1	0,5	0,3	0,1	1	1				0,5	1
<i>Dosinia exoleta</i>	17	1			1	133,334	1	1		0,8	1	0,1	1		1			1
<i>Echinocyamus pusillus</i>	17	1			1	1069,340	1	1		0,3	0,3	1	0					
<i>Galathea intermedia</i>	17	1			1	3,000	1	1		0,8	0,5	1	1					1
<i>Goniadella bobretzkii</i>	17	1			1	172,666	1				0,1	1			1			
<i>Liocarcinus</i> sp.			16		1		1			1	1	1			1			
<i>Lithothamnion sonderi</i> & <i>Phymatolithon</i> (encrusting calcareous red algae)			16		1		1	1	1	0,5	1	1	1	1				1
Paguridae			16		1		1	0,5		1	1	1						
<i>Pagurus cuanensis</i>	17	1		1		0,125	1	0,5		1	1	1	1					
<i>Pododesmus</i> sp.			16		1		1	1		0,5	0,3	1	1					1
<i>Polititapes rhomboides</i>	17	1		1		0,250	1	1		0,5	0,8	0,5	1					
Porifera (large structure forming species)			16		1		1	1		1	0,3	1				1		1
<i>Protodorrvillea kefersteini</i>	17	1			1	226,880	1				0,1	1			1			
<i>Sabellaria spinulosa</i>	17	1		1		0,278	1	1		0,5	0,1	1	1	0,5	0,5			1
<i>Simnia patula</i>	17	1		1		0,059	1	1			0,1	1	1					1
<i>Spiophanes kroyeri</i>	17	1			1	13,526	1	1		0,3	0,1	1				1		
<i>Spirobranchus triqueter</i>	17	1			1	29,334	1			0,5	1	1	1		1			
<i>Terebellides stroemi</i>	17	1			1	13,076	1	1		0,5	0,3	0,5				1		
<i>Timoclea ovata</i>	17	1			1	17,112	1	1		0,3	0,8	0,5						
<i>Upogebia deltaura</i>	17	1			1	540,660	1		1	1	0,3	1				1	1	
<i>Urothoe marina</i>	17	1			1	493,560	1			0,3	0,2	1	1	1				
<i>Urticina</i> sp.			16		1		1	1		1	1	0,5	1					1
Number of species (S)	21	21	9				30	23	4	26	30	30	16	6	7	2	14	

* Results of Hamon grabs and Boxcores are combined in the evaluation (without compensation of the slight difference in sampled surface area).

The specific evaluation to identify the possible impact of ecological disturbance as the cause of changes in the quality status (B.) and the specific evaluation to indicate changes in the biological activation of the sea floor top layer (I.) by taking bioturbating/ bioirrigating species into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Xandarovula patula = *Simnia patula*; *Polititapes virgineus* = *Polititapes rhomboides*; *Tapes rhomboides* = *Polititapes rhomboides*; *Venerupis rhomboides* = *Polititapes rhomboides*; *Pomatoceros*

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triqueter = *Spirobranchus triqueter*; *Ceriantharia* (not identified to species level in current monitoring) = *Cerianthus loydii*; Sabellidae (not identified to species level in current monitoring) = *Chone duner*; Corallinaceae = *Lithothamnion sonderi* & *Phymatolithon* (encrusting calcareous red algae); Paguridae = *Pagurus bernhardus* + *P. cuanensis*. The typical species '*Pododesmus patelliformis*' for Habitat Directive habitat H1170 has been replaced by '*Pododesmus* sp.' as the species cannot be identified on basis of video (and natural densities are too low for the Hamon grab).

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Appendix 1c. Reference list of indicator species for the area with special ecological value Central Oystergrounds.

ASEV Centrale Oestergronden	Expected number of samples per year (2015+)		Data type of evaluation		R_i	IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning		
	Boxcore (0,078 m ²)	Dredge (20m ²)	Hit rate (spatial)	Densities (number/m ²)		General quality	A	B	C	D			G	H	I
Indicator Species															
<i>Acanthocardia echinata</i>		12		1	0,450	1	1		1	1	0,1	1			
<i>Amphiura filiformis</i>	18			1	1331,183	1	0,5	1	0,5	1	1	0,5	1	1	1
<i>Aphrodite aculeata</i>		12		1	0,716	1	0,5		0,8	1	0,1	1			
<i>Arctica islandica</i>		12		1	0,306	1	1	0,5	1	1	0,1				
<i>Brissopsis lyrifera</i>		12		1	5,734	1	1	1	0,8	0,5	1	1			1
<i>Callianassa subterranea</i>	18			1	123,917	1	0,5	0,5	0,8	0,2	1	0,5	1	1	1
<i>Chaetoperus variopedatus</i>	18			1	30,483	1	1		0,8	0,3	0,1	1		1	
<i>Chamelea striatula</i>		12		1	4,714	1	1		0,8	1	0,5				
<i>Corbula gibba</i>	18			1	1209,400	1	0,5		0,5	0,2	1	1	1		
<i>Cylichna cylindracea</i>	18			1	60,000	1			0,3	0,3	0,1	1			
<i>Dosinia lupinus</i>		12		1	0,900	1	1		0,8	1	0,5	0,5			
<i>Echinocardium cordatum</i>		12		1	39,375	1		1	0,8	1	1		1		1
<i>Echinocardium flavescens</i>		12		1	1,756	1			0,8	1	0,5	1			1
<i>Nephtys incisa</i>	18			1	21,940	1	0,5	1	0,5	0,5	1	1			
<i>Nucula nitidosa</i>	18			1	136,734	1	1		0,5	1	1	0,5			
<i>Sthenelais limicola</i>	18			1	56,333	1	1	1	0,5	0,3	1				
<i>Terebellides stroemi</i>	18			1	23,517	1	1		0,5	0,3	0,5	1		1	
<i>Turritella communis</i>		12		1	27,956	1	1		0,5	1	1	1			
<i>Upogebia deltaura</i>		12		1	1,509	1	1		1	0,3	1	1	1	1	1
<i>Upogebia stellata</i>		12		1	0,678	1	1		0,8	0,3	1	1	1	1	1
Number of species (S)	9	11				20	17	7	20	20	20	16	6	6	7

Nucula turgida = *Nucula nitidosa*; *Chamelea gallina* = *Chamelea striatula*

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Appendix 1d. Reference list of indicator species for the area with special ecological value Frisian Front.

ASEV Friese Front	Expected number of samples per year (2015+)		Data type of evaluation		R _i		IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning		
	Boxcore (0,078 m ²)	Dredge (20m ²)	Hit rate (spatial)	Densities (number/m ²)	Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (20 m ²); densities (n/m ²)	General quality	A	B	C	D			G	H	I
Indicator Species																
<i>Amphiura filiformis</i>	9			1	1749,820		1	0,5	1	0,5	1	1	0,5	1	1	1
<i>Atherospio guillei</i>	9			1	75,313		1			0,3	0,2	1	1			
<i>Callianassa subterranea</i>	9			1	293,250		1	0,5	0,5	0,8	0,2	1	0,5	1	1	1
<i>Corystus cassivelaunus</i>		15		1		0,646	1	1	1	1	1	1	0,5	1		1
<i>Dosinia lupinus</i>		15		1		1,421	1	1		0,8	1	0,1	0,5			
<i>Euspira pulchella</i>	9			1	38,450		1	1		0,5	0,3	1				
<i>Echinocardium cordatum</i>		15		1		36,914	1		1	0,5	1	1				1
<i>Goneplax rhomboides</i>		15		1		4,714	1	1		1	0,5	1	1	1	1	1
<i>Leptosynapta inhaerens</i>	9			1	19,226		1	1		0,5	0,3	0,1	0,5			1
<i>Nephtys incisa</i>	9			1	22,425		1	0,5	1	0,3	0,5	1	0,5			
<i>Oxydromus flexuosus</i>	9			1	25,613		1			0,3	0,2	1	1	0,5		
<i>Ophiura albida</i>		15		1		39,375	1	0,5	1	0,5	0,3	1	0,5	1		0,5
<i>Podarkeopsis helgolandica</i> /	9			1	35,250		1			0,3	0,2	1	1			
<i>Thracia convexa</i>		15		1		0,585	1	1	1	0,8	1	0,1	1			
<i>Upogebia deltaura</i>		15		1		23,392	1	1		1	0,3	1	1	1	1	1
<i>Upogebia stellata</i>		15		1		0,488	1	1		0,8	0,3	1	0,5	1	1	1
Number of species (S)	8	8					16	12	7	16	16	16	14	8	5	9

Euspira poliana = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*;
Polydora guillei = *Atherospio guillei*; *Ophiodromus flexuosus* = *Oxydromus flexuosus*.

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Appendix 1e. Reference list of indicator species for the area with special ecological value Brown Bank.

ASEV Bruine Bank	Expected number of samples per year (2015+)		Data type of evaluation		R_i		IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning		
	Boxcore (0,078 m ²)	Dredge (20m ²)	Hit rate (spatial)	Densities (number/m ²)			General quality	A	B	C	D			G	H	I
Indicator Species					Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (20 m ²); densities (n/m ²)										
<i>Angulus fabula</i>	7			1	17,560		1	1		0,5	0,3	1				
<i>Bathyporeia elegans</i>	7			1	246,775		1		1	0,3	0,1	1		1		1
<i>Bathyporeia guilliamsoniana</i>	7			1	84,880		1		1	0,3	0,1	1		1		1
<i>Corystus cassivelaunus</i>		9		1		0,485	1	1	1	1	1	1	0,5	1		1
<i>Donax vittatus</i>		9		1		1,520	1	1		0,5	0,3	1	1	1		
<i>Echinocardium cordatum</i>		9		1		4,601	1		1	0,8	1	1		1		1
<i>Echinocyamus pusillus</i>	7			1	5,667		1		1	0,3	1	1				
<i>Ensis ensis</i>		9		1		1,172	1	1		1	1	0,1	1	1		
<i>Ensis siliqua</i>		9		1		0,248	1	1		1	1	0,1	0,5	1		
<i>Euspira pulchella</i>		9		1		0,954	1	0,5		0,5	0,3	1		1		
<i>Goniada maculata</i>	7			1	10,975		1				0,1	1		1		
<i>Lanice conchilega</i>	7			1	40,250		1	0,5	1	0,5	0,1	1		1	1	1
<i>Liocarcinus holsatus</i>		9		1		0,251	1			1	1	1		1		
<i>Nephtys cirrosa</i>	7			1	289,700		1	0,5	1	0,5	0,5	1	1			
<i>Ophiura albida</i>		9		1		8,342	1	0,5	1	0,5	0,3	1	0,5	1		0,5
<i>Ophiura ophiura</i>		9		1		2,525	1	0,5	1	0,5	0,5	1	0,5	1		
<i>Pagurus bernhardus</i>		9		1		0,488	1	0,5		1	1	1				
<i>Spiophanes bombyx</i>	7			1	819,320		1			0,3	0,2	1				1
<i>Spisula elliptica</i>		9		1		1,565	1	1		0,8	0,3	1	0,5	1		1
<i>Thia scutellata</i>		9		1		1,253	1	0,5		0,5	0,5	1	1			
<i>Urothoe poseidonis</i>	7			1	169,850		1			0,3	0,1	1		0,5		
Number of species (S)	9	12					21	13	9	20	21	21	9	15	1	8

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Ophiura texturata* = *Ophiura ophiura*; *Eupagurus bernhardus* = *Pagurus bernhardus*.

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Appendix 1f. Reference list of indicator species for the area with special ecological value North Sea Coastal Zone.

ASEV Noordzeekustzone	number of samples per year (2015+)		Data type of evaluation	R_i			IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning			Habitat Directive
	Boxcore (0,078 m ²)	Dredge (15 m ²)					General quality	A	B	C	D			G	H	I	J
Indicator Species			Hit rate (spatial)	Densities (number/m ²)	Boxcore (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (15 m ²); densities (n/m ²)											
<i>Abra alba</i>	16			1	147,470		1	1	1	1	1	1	0,5	1		1	1
<i>Angulus fabula</i>	16			1	523,780		1	1		0,5	0,3	1	1				1
<i>Bathyporeia elegans</i>	16			1	200,390		1		1	0,25	0,1	1		1		1	1
<i>Buccinum undatum</i>		66		1		0,030	1	1	1	1	1						1
<i>Chamelea striatula</i>		66		1		0,379	1	1		0,75	1	0,5					
<i>Corystes cassivelaunus</i>		66		1		0,510	1	1	1	1	1	1		1		1	
<i>Donax vittatus</i>		66		1		4,477	1	1		0,5	0,3	1		1			1
<i>Echinocardium cordatum</i>	16			1	85,470		1		1	0,75	1	1		1		1	1
<i>Euspira pulchella</i>		66		1		0,685	1			0,5	0,3	1		1			1
<i>Lanice conchilega</i>	16			1	1968,860		1	0,5	1	0,5	0,1	1		1	1	1	1
<i>Liocarcinus holsatus</i>		66		1		2,486	1			1	1	1		1			1
<i>Lutraria lutraria</i>		66		1		0,541	1	0,5		1	0,5	1					
<i>Macoma balthica</i>		66		1		65,978	1			0,5	1	1	1	1			1
<i>Mactra stultorum</i>		66		1		0,068	1	1		0,75	0,6	1					1
<i>Magelona johnstoni</i> + <i>M. filiformis</i>	16			1	4711,040		1			0,25	0,3	1		1		1	1
<i>Mytilus edulis</i>		66		1		0,078	1	0,5		0,5	0,5	1		1	1		
<i>Nephtys cirrosa</i>	16			1	188,090		1	1	1	0,5	0,5	1					1
<i>Nephtys hombergii</i>	16			1	105,340		1	1	1	0,5	0,5	1					1
<i>Ophiura ophiura</i>		66		1		26,869	1	0,5	1	0,5	0,5	1	0,5	1			1
<i>Owenia fusiformis</i>	16			1	47,850		1		1	0,25	0,5	1		1	1	1	
<i>Pagurus bernhardus</i>		66		1		1,814	1	0,5		1	1	1					1
<i>Pontocrates altamarinus</i>	16			1	97,400		1	0,5		0,25	0,1	1		1			1
<i>Spiophanes bombyx</i>	16			1	750,330		1			0,25	0,2	1				1	1
<i>Spisula subtruncata</i>		66		1		246,470	1	1		0,75	0,3	1	0,5	1		1	1
<i>Urothoe poseidonis</i>	16			1	838,340		1			0,25	0,1	1		0,5			1
Number of species (S)	11	12					25	16	10	25	25	24	5	16	3	9	20

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Mactra corallina* = *Mactra stultorum*; *Ophiura texturata* = *Ophiura ophiura*; *Magelona papillicornis* = *M. johnstoni* + *M. filiformis* (as *M. papillicornis* has never been present in the North Sea); *Chamelea gallina* = *Chamelea striatula* (as *C. gallina* is not present in the Dutch North Sea).

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Appendix 1g. Reference list of indicator species for the area with special ecological value Voordelta.

ASEV Voordelta	Expected number of samples per year (2015+)		Data type of evaluation		R_i		IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning			Habitat Directive	
	Boxcore (0,078 m ²)	Dredge (15 m ²)	Hit rate (spatial)	Densities (number/m ²)	Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (15 m ²); densities (n/m ²)	General quality	A	B	C	D			G	H	I		J
Indicator Species																		
<i>Abra alba</i>		83		1		222,260	1	1	1	1	1	1	0,5	1		1		
<i>Angulus fabula</i>	16			1	223,370		1	1		0,5	0,3	1	1			1		
<i>Bathyporeia elegans</i>	16			1	149,690		1		1	0,25	0,1	1		1		1		
<i>Buccinum undatum</i>		83		1		0,024	1	1	1	1	1					1		
<i>Donax vittatus</i>		83		1		7,190	1	1		0,5	0,3	1		1		1		
<i>Echinocardium cordatum</i>	16			1	61,880		1		1	0,75	1	1		1		1		
<i>Euspira pulchella</i>	16			1	56,170		1			0,5	0,3	1		1		1		
<i>Lanice conchilega</i>	16			1	2871,900		1	0,5	1	0,5	0,1	1		1	1	1		
<i>Liocarcinus holsatus</i>		83		1		0,920	1			1	1	1		1		1		
<i>Lutraria lutraria</i>		83		1		0,300	1	0,5		1	0,5	1						
<i>Macoma balthica</i>		83		1		383,940	1			0,5	1	1	1	1		1		
<i>Mactra stultorum</i>		83		1		0,060	1	1		0,75	0,6	1				1		
<i>Magelona johnstoni</i> + <i>M. filiformis</i>	16			1	2945,130		1			0,25	0,3	1		1		1		
<i>Nephtys cirrosa</i>	16			1	111,570		1	1	1	0,5	0,5	1				1		
<i>Nephtys hombergii</i>	16			1	145,300		1	1	1	0,5	0,5	1				1		
<i>Ophiura ophiura</i>		83		1		32,840	1	0,5	1	0,5	0,5	1	0,5	1		1		
<i>Owenia fusiformis</i>	16			1	128,200		1		1	0,25	0,5	1		1	1	1		
<i>Pagurus bernhardus</i>		83		1		2,160	1	0,5		1	1	1				1		
<i>Pontocrates altamarinus</i>	16			1	26,340		1	0,5		0,25	0,1	1		1		1		
<i>Spiophanes bombyx</i>	16			1	3705,200		1			0,25	0,2	1			1	1		
<i>Spisula subtruncata</i>		83		1		31,606	1	1		0,75	0,3	1	0,5	1		1		
<i>Thia scutellata</i>		83		1		0,019	1	0,5		0,5	0,5	1	1					
<i>Urothoe poseidonis</i>	16			1	1029,130		1			0,25	0,1	1		0,5		1		
Number of species (S)	12	11					23	14	9	23	23	22	6	14	2	20		

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Mactra corallina* = *Mactra stultorum*; *Ophiura texturata* = *Ophiura ophiura*; *Magelona papillicornis* = *M. johnstoni* + *M. filiformis* (as *M. papillicornis* has never been present in the North Sea).

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Appendix 1h. Reference list of indicator species for the area with special ecological value Vlakte van de Raan.

ASEV Vlakte van de Raan	Expected number of samples per year (2015+)		Data type of evaluation		R_i		IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning			Habitat Directive
Indicator Species	Boxcore (0,078 m ²)	Dredge (15 m ²)	Hit rate (spatial)	Densities (number/m ²)	Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (15 m ²); densities (n/m ²)	General quality	A	B	C	D			G	H	I	J
<i>Abra alba</i>		39		1		64,480	1	1	1	1	1	1	0,5	1		1	1
<i>Angulus fabula</i>	8			1	40,000		1	1		0,5	0,3	1	1				1
<i>Bathyporeia elegans</i>	8			1	68,000		1		1	0,25	0,1	1		1		1	1
<i>Buccinum undatum</i>		39		1		0,030	1	1	1	1	1						1
<i>Donax vittatus</i>		39		1		6,440	1	1		0,5	0,3	1		1			1
<i>Echinocardium cordatum</i>	8			1	6,800		1		1	0,75	1	1		1		1	1
<i>Euspira pulchella</i>	8			1	4,000		1			0,5	0,3	1		1			1
<i>Lanice conchilega</i>	8			1	264,810		1	0,5	1	0,5	0,1	1		1	1	1	1
<i>Liocarcinus holsatus</i>		39		1		0,850	1			1	1	1		1			1
<i>Lutraria lutraria</i>		39		1		0,140	1	0,5		1	0,5	1					
<i>Macoma balthica</i>		39		1		320,520	1			0,5	1	1	1	1			1
<i>Mactra stultorum</i>		39		1		0,050	1	1		0,75	0,6	1					1
<i>Magelona johnstoni</i> + <i>M. filiformis</i>	8			1	520,000		1			0,25	0,3	1		1		1	1
<i>Nephtys cirrosa</i>	8			1	86,000		1	1	1	0,5	0,5	1					1
<i>Nephtys hombergii</i>	8			1	37,400		1	1	1	0,5	0,5	1					1
<i>Ophiura ophiura</i>		39		1		30,440	1	0,5	1	0,5	0,5	1	0,5	1			1
<i>Owenia fusiformis</i>	8			1	16,000		1		1	0,25	0,5	1		1	1	1	
<i>Pagurus bernhardus</i>		39		1		2,010	1	0,5		1	1	1					1
<i>Pontocrates altamarinus</i>	8			1	12,750		1	0,5		0,25	0,1	1		1			1
<i>Spiophanes bombyx</i>	8			1	648,000		1			0,25	0,2	1				1	1
<i>Spisula subtruncata</i>		39		1		16,120	1	1		0,75	0,3	1	0,5	1		1	1
<i>Urothoe poseidonis</i>	8			1	120,000		1			0,25	0,1	1		0,5			1
Number of species (S)	12	10					22	13	9	22	22	21	5	14	2	8	20

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Mactra corallina* = *Mactra stultorum*; *Ophiura texturata* = *Ophiura ophiura*; *Magelona papillicornis* = *M. johnstoni* + *M. filiformis* (as *M. papillicornis* has never been present in the North Sea).

Appendix 1i. Reference list of indicator species for the EUNIS 3 ecotope 'Deep coarse sediment'.

EUNIS ecotope: Deep coarse sediment (Diep grof sediment)	Expected number of samples per year (2015+)			Data type of evaluation		R _i		IV for Pressure					IV for recovery		National importance		IV for Ecological Functioning		
	Hamon grab (0,09 m ²)	Boxcore (0,078 m ²)	Video tracks (20 m ²)	Hit rate (spatial)	Densities (number/m ²)	Hamon grab (0,09 m ²); hit rate (per grab) or density (n/m ²)*	Video tracks (20 m ²); densities (n/m ²)	General quality	A	B	C	D	E	F	G	H	I		
Indicator Species																			
Arcopagia crassa	10	1			1	0,526		1	1		0,5	1	0,5	1					
Aequipecten opercularis			10		1	0,016		1	1		1	0,6	1	1					
Alcyonium digitatum			10		1	18,042		1	1		1	1	0,5	1					
Aonides paucibranchiata	10	1			1	1224,360		1				0,1	1		1				
Aporrhais pespelecani	10	1		1		0,125		1	1		0,8	0,3	1	1					
Arctica islandica	10	1		1		0,250		1	1		1	1	0,1						
Buccinum undatum			10		1	0,382		1	1	1	1	1	0,1						
Cerianthus lloydii	10	1			1	92,364		1	1		1	1	0,1			1			
Chone duneri	10	1			1	217,350		1	1	0,5	0,3	0,1	1	1			0,5		
Dosinia exoleta	10	1			1	129,454		1	1		0,8	1	0,1	1					
Echinocyamus pusillus	10	1			1	1438,540		1	1		0,3	0,3	1						
Galathea intermedia	10	1			1	3,000		1	1		0,8	0,5	1	1					
Goniadella bobrezkii	10	1			1	129,090		1				0,1	1		1				
Lithothamnion sonderi & Phymatolithon (encrusting calcareous red algae)			10		1	0,222		1	1	1	0,5	1	1	1					
Pagurus cuanensis	10	1		1		0,250		1	0,5		1	1	1	1					
Pododesmus sp			10		1	0,005		1	1		0,5	0,3	1	1					
Polititapes rhomboides	10	1		1		0,250		1	1		0,5	0,8	0,5	1					
Porifera (large structure forming species)			10		1	0,004		1	1		1	0,3	1			1			
Protodorvillea kefersteini	10	1			1	322,920		1				0,1	1		1				
Sabellaria spinulosa	10	1		1		0,278		1	1		0,5	0,1	1	1	0,5	0,5			
Simnia patula	10	1		1		0,059		1	1			0,1	1	1					
Spiophanes kroyeri	10	1			1	13,526		1	1		0,3	0,1	1			1			
Spirobranchus triqueter	10	1			1	32,000		1			0,5	1	1	1		1			
Terebellides stroemi	10	1			1	13,076		1	1		0,5	0,3	0,5			1			
Timoclea ovata	10	1			1	3,250		1	1		0,3	0,8	0,5						
Upogebia deltaura	10	1			1	593,820		1		1	1	0,3	1			1	1		
Urothoe marina	10	1			1	694,540		1			0,3	0,2	1	1	1				
Urticina sp			10		1	0,572		1	1		1	1	0,5	1					
Number of species (S)	21	21	7					28	22	4	24	28	28	16	5	7	2		

* Results of Hamon grabs and Boxcores are combined in the evaluation (without compensation of the slight difference in sampled surface area).

The specific evaluation to identify the possible impact of ecological disturbance as the cause of changes in the quality status (B) and the specific evaluation to indicate changes in the biological activation of the sea floor top layer (I.) by taking bioturbating/bioirrigating species into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Xandarovula patula = *Simnia patula*; *Polititapes virgineus* = *Polititapes rhomboides*; *Tapes rhomboides* = *Polititapes rhomboides*; *Venerupis rhomboides* = *Polititapes rhomboides*; *Pomatoceros triqueter* = *Spirobranchus triqueter*; *Ceriantharia* (not identified to species level in current monitoring) = *Cerianthus lloydii*; *Sabellidae* (not identified to species level in current monitoring) = *Chone duneri*; *Corallinaceae* = *Lithothamnion sonderi* &

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Phymatolithon (encrusting calcareous red algae); *Paguridae* = *Pagurus bernhardus* + *P. Cuanensis*. The typical species '*Pododesmus patelliformis*' for Habitat Directive habitat H1170 has been replaced by '*Pododesmus* sp.' as the species cannot be identified on basis of video (and natural densities are too low for the Hamon grab).

Appendix 1j. Reference list of indicator species for the EUNIS 3 ecotope 'Deep sandy substratum'.

EUNIS ecotope: Deep sandy (Diep zandig) [#]	Expected number of samples per year (2015+)				Data type of evaluation		R _i	IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning				
Indicator Species	Boxcore (0,078 m ²)	Hamon grab (0,09 m ²)	Dredge (20m ²)	Video tracks (20 m ²)	Hit rate (spatial)	Densities (number/m ²)		Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (20 m ²); densities (n/m ²)	General quality	A	B			C	D	E	F	G
<i>Angulus fabula</i>	45	4				1	64,010		1	1		0,5	0,3	1					
<i>Arctica islandica</i>			12	9		1		0,024	1	1	0,5	1	1	0,1					
<i>Bathyporeia elegans</i>	45	4				1	100,220		1		1	0,3	0,1	1			1		1
<i>Bathyporeia guilliamsoniana</i>	45	4				1	89,613		1		1	0,3	0,1	1			1		1
<i>Corystus cassivelaunus</i>			12	9		1		0,485	1	1	1	1	1	1	0,5	1			1
<i>Donax vittatus</i>			12	9		1		1,520	1	1		0,5	0,3	1	1	1			
<i>Dosinia lupinus</i>	45	4				1	9,167		1	1		0,8	1	0,5	0,5				
<i>Echinocardium cordatum</i>	45	4				1	45,154		1		1	0,8	1	1			1		1
<i>Echinocyamus pusillus</i>	45	4				1	158,000		1		1	0,3	1	1					
<i>Ensis ensis</i>			12	9		1		1,172	1	1		1	1	0,1	1	1			
<i>Ensis siliqua</i>			12	9		1		0,248	1	1		1	1	0,1	0,5	1			
<i>Euspira pulchella</i>	45	4				1	31,890		1	0,5		0,5	0,3	1			1		
<i>Gari fervensis</i>			12	9		1		0,592	1	1		0,8	1	0,5	1				
<i>Goniada maculata</i>	45	4				1	26,297		1				0,1	1			1		
<i>Lanice conchilega</i>	45	4				1	166,790		1	0,5	1	0,5	0,1	1			1	1	1
<i>Liocarcinus holsatus</i>			12	9		1		0,126	1			1	1	1			1		
<i>Nephtys cirrosa</i>	45	4				1	67,670		1	0,5	1	0,5	0,5	1	1				
<i>Ophiura albida</i>			12	9		1		4,171	1	0,5	1	0,5	0,3	1	0,5	1			0,5
<i>Ophiura ophiura</i>			12	9		1		1,262	1	0,5	1	0,5	0,5	1	0,5	1			
<i>Pagurus bernhardus</i>			12	9		1		0,488	1	0,5		1	1	1					
<i>Psammechinus miliaris</i>			12	9		1		0,211	1	0,5	1	0,8	1	1	1				
<i>Spiophanes bombyx</i>	45	4				1	634,960		1			0,3	0,2	1					1
<i>Spisula elliptica</i>			12	9		1		0,783	1	1		0,8	0,3	1	0,5	1			1
<i>Thia scutellata</i>			12	9		1		0,627	1	0,5		0,5	0,5	1	1				
<i>Urothoe poseidonis</i>	45	4				1	89,924		1			0,3	0,1	1			0,5		
Number of species (S)	12	12	13	13					25	17	11	24	25	25	12	15	1		8

[#] As Dredge samples are only taken inside nature areas at sea, it should be tested on basis of boxcore samples if areas inside and outside ASEV are similar (no significant differences); if so, dredge samples can be included in the evaluation (as in the shown table) as well.

*Results of Hamon grabs and Boxcores are combined in the evaluation (without compensation of the slight difference in sampled surface area). As data collected using video deviate too much from data collected using a dredge, video data are not used in the evaluation.

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Ophiura texturata* = *Ophiura ophiura*; *Eupagurus bernhardus* = *Pagurus bernhardus*.

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Appendix 1k. Reference list of indicator species for the EUNIS 3 ecotope 'Deep muddy substratum'.

EUNIS ecotope: Deep muddy (Diep slibrijk) [#]	Expected number of samples per year (2015+)				Data type of evaluation		R_i		IV for Pressure					IV for Ecological Functioning				
Indicator Species	Boxcore (0,078 m ²)	Hamon grab (0,09 m ²)	Dredge (20m ²)	Video tracks (20 m ²)	Hit rate (spatial)	Densities (number/m ²)	Boxcorer (0,078 m ²) + Hamon grab (0,09 m ²); hit rate (per core/grab) or densities (n/m ²)*	Dredge + video track (20 m ²); densities (n/m ²)	General quality	A	B	C	D	E	F	G	H	I
<i>Acanthocardia echinata</i>			24	5		1		0,248	1	1		1	1	0,1	1			
<i>Amphiura filiformis</i>	31	3				1	1166,070		1	0,5	1	0,5	1	1	0,5	1	1	1
<i>Aphrodite aculeata</i>			24	5		1		0,716	1	0,5		0,8	1	0,1	1			
<i>Arctica islandica</i>			24	5		1		0,602	1	1	0,5	1	1	0,1				
<i>Atherospio guillei</i>	31	3				1	33,058		1			0,3	0,2	1	1			
<i>Brissopsis lyrifera</i>			24	5		1		2,945	1	1	1	0,8	0,5	1	1			1
<i>Callianassa subterranea</i>	31	3				1	201,079		1	0,5	0,5	0,8	0,2	1	0,5	1	1	1
<i>Chaetopterus variopedatus</i>	31	3				1	51,220		1	1		0,8	0,3	0,1	1		1	
<i>Chamelea striatula</i>	31	3				1	11,547		1	1		0,8	1	0,5				
<i>Corbula gibba</i>	31	3				1	477,063		1	0,5		0,5	0,2	1	1	1		
<i>Corystus cassivelaunus</i>			24	5		1		0,684	1	1	1	1	1	1	0,5	1		1
<i>Cylichna cylindracea</i>	31	3				1	32,200		1			0,3	0,3	0,1	1			
<i>Dosinia lupinus</i>			24	5		1		0,900	1	1		0,8	1	0,5	0,5			
<i>Echinocardium cordatum</i>	31	3				1	80,942		1		1	0,8	1	1		1		1
<i>Echinocardium flavescens</i>			24	5		1		0,927	1			0,8	1	0,5	1			1
<i>Euspira pulchella</i>	31	3				1	23,105		1	1		0,5	0,3	1				
<i>Goneplax rhomboides</i>			24	5		1		4,714	1	1		1	0,5	1	1	1	1	1
<i>Leptosynapta inhaerens</i>	31	3				1	14,620		1	1		0,5	0,3	0,1	0,5			1
<i>Nephtys incisa</i>	31	3				1	21,940		1	0,5	1	0,5	0,5	1	1			
<i>Nucula nitidosa</i>	31	3				1	122,800		1	1		0,5	1	1	0,5			
<i>Ophiura albida</i>			24	5		1		39,375	1	0,5	1	0,5	0,3	1	0,5	1		0,5
<i>Oxydromus flexuosus</i>	31	3				1	20,226		1			0,3	0,2	1	1	0,5		
<i>Podarkeopsis helgolandica</i> / <i>P. capensis</i>	31	3				1	24,880		1			0,3	0,2	1	1			
<i>Sthenelais limicola</i>	31	3				1	10,795		1	1	1	0,5	0,3	1				
<i>Terebellides stroemi</i>	31	3				1	9,442		1	1		0,5	0,3	0,5	1		1	
<i>Thracia convexa</i>			24	5		1		0,291	1	1	1	0,8	1	0,1	1			
<i>Turritella communis</i>			24	5		1		19,108	1	1		0,5	1	1	1			
<i>Upogebia deltaura</i>	31	3				1	43,900		1	1		1	0,3	1	1	1	1	1
<i>Upogebia stellata</i>			24	5		1		0,588	1	1		0,8	0,3	1	1	1	1	1
Number of species (S)	17	17	12	12					29	23	10	29	29	29	24	10	7	11

[#] As Dredge samples are only taken inside nature areas at sea, it should be tested on basis of Boxcore samples if areas inside and outside ASEVs are similar (no significant differences); if so, dredge samples can be included in the evaluation (as in the shown table) as well.

*Results of Hamon grabs and Boxcores are combined in the evaluation (without compensation of the slight difference in sampled surface area). As data collected using video deviate too much from data collected using a dredge, video data are not used in the evaluation.

Nucula turgida = *Nucula nitidosa*; *Chamelea gallina* = *Chamelea striatula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Polydora guillei* = *Atherospio guillei*; *Ophiodromus flexuosus* = *Oxydromus flexuosus*.

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Appendix 11. Reference list of indicator species for the EUNIS 3 ecotope 'Shallow to moderate deep coarse sediment'.

EUNIS ecotope: Shallow coarse sediment (Ondiep grof sediment)	Expected number of samples per year (2015+)		Type of evaluation		R_i		IV for Pressure					IV for recovery		National importance	IV for Ecological Functioning		
	Boxcore (0,078 m ²)	Dredge (20m ²)	Hit rate (spatial)	Densities (number/m ²)	Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (20 m ²); densities (n/m ²)	General quality	A	B	C	D						
Indicator Species																	
<i>Abra alba</i>	27			1	22,060		1	1	1	1	1	1			1		1
<i>Alcyonium digitatum</i>		13		1		0,077	1	1		1	1	0,5	0,5				
<i>Angulus fabula</i>	27			1	313,037		1	1		0,5	0,3	1					
<i>Bathyporeia guilliamsoniana</i>	27			1	185,088		1		1	0,25	0,1	1	0,5	1			
<i>Chamelea striatula</i>		13		1		1,935	1	1		0,75	1	0,5					
<i>Corystes cassivelaunus</i>		13		1		0,138	1	1	1	1	1	1		1		1	1
<i>Echinocardium cordatum</i>	27			1	90,267		1		1	0,75	1	1		1		1	1
<i>Echinocyamus pusillus</i>	27			1	95,685		1		1	0,25	1	1	0,5				
<i>Euspira pulchella</i>	27			1	151,053		1			0,5	0,3	1		1			
<i>Liocarcinus holsatus</i>		13		1		3,384	1			1	1	1		1			
<i>Lutraria lutraria</i>		13		1		5,468	1	0,5		1	0,5	1					
<i>Ophiura ophiura</i>		13		1		18,390	1	1	1	0,5	0,5	1		1			
<i>Pagurus bernhardus</i>		13		1		1,743	1	0,5		1	1	1					
<i>Thia scutellata</i>	27			1	15,574		1	0,5		0,5	0,5	1	1		1		
Number of species (S)	7	7					14	9	6	14	14	14	4	7	0	4	4

The specific evaluation to identify the possible impact of changes at the national level (F.) by taking characteristic species into account, to indicate changes in habitat structure (H.) by taking species creating permanent structures into account, and to indicate possible impact on biological activation of the sea floor (I.) by taking bioturbating and bioirrigating species into account, does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Ophiura texturata* = *Ophiura ophiura*; *Eupagurus bernhardus* = *Pagurus bernhardus*.

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Appendix 1m. Reference list of indicator species for the EUNIS 3 ecotope 'Shallow to moderate deep sandy substratum'.

EUNIS ecotope: Shallow sandy (Ondiep zandig)	Expected number of samples per year (2015+)		Data type of evaluation		R_i	IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning		
	Boxcore (0,078 m ²)	Dredge (15 m ²)	Hit rate (spatial)	Densities (number/m ²)		General quality	A	B	C	D			G	H	I
Indicator Species					Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)										
<i>Abra alba</i>		224		1	94,666	1	1	1	1	1	1	0,5	1		1
<i>Angulus fabula</i>	56			1	402,834	1	1		0,5	0,3	1	1			
<i>Bathyporeia elegans</i>	56			1	328,316	1		1	0,25	0,1	1		1		1
<i>Buccinum undatum</i>		224		1	0,012	1	1	1	1	1					
<i>Chamelea striatula</i>		224		1	4,098	1	1		0,75	1	0,5				
<i>Corystes cassivelaunus</i>		224		1	0,235	1	1	1	1	1	1		1		1
<i>Donax vittatus</i>		224		1	3,647	1	1		0,5	0,3	1		1		
<i>Echinocardium cordatum</i>	56			1	111,872	1		1	0,75	1	1		1		1
<i>Euspira pulchella</i>	56			1	69,230	1			0,5	0,3	1		1		
<i>Lanice conchilega</i>	56			1	2082,360	1	0,5	1	0,5	0,1	1		1	1	1
<i>Liocarcinus holsatus</i>		224		1	1,904	1			1	1	1		1		
<i>Lutraria lutraria</i>		224		1	2,544	1	0,5		1	0,5	1				
<i>Mactra stultorum</i>		224		1	0,148	1	1		0,75	0,6	1				
<i>Magelona johnstoni</i> + <i>M. filiformis</i>	56			1	3223,600	1			0,25	0,3	1		1		1
<i>Mytilus edulis</i>		224		1	49,819	1	0,5		0,5	0,5	1		1	1	
<i>Nephtys cirrosa</i>	56			1	261,444	1	1	1	0,5	0,5	1				
<i>Ophiura ophiura</i>		224		1	15,856	1	0,5	1	0,5	0,5	1	0,5	1		
<i>Owenia fusiformis</i>	56			1	162,643	1		1	0,25	0,5	1		1	1	1
<i>Pagurus bernhardus</i>		224		1	1,390	1	0,5		1	1	1				
<i>Pontocrates altamarinus</i>	56			1	66,768	1	0,5		0,25	0,1	1		1		
<i>Sigalion mathildae</i>	56			1	50,939	1	0,5		0,5	0,4	1	1			
<i>Spiophanes bombyx</i>	56			1	3553,400	1			0,25	0,2	1				1
<i>Spisula subtruncata</i>		224		1	72,775	1	1		0,75	0,3	1	0,5	1		1
<i>Urothoe poseidonis</i>	56			1	1249,887	1			0,25	0,1	1		0,5		
Number of species (S)	12	12				24	16	9	24	24	23	5	15	3	9

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Mactra corallina* = *Mactra stultorum*; *Ophiura texturata* = *Ophiura ophiura*; *Magelona papillicornis* = *M. johnstoni* + *M. filiformis* (as *M. papillicornis* has never been present in the North Sea); *Chamelea gallina* = *Chamelea striatula* (as *C. gallina* is not present in the Dutch North Sea).

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Appendix 1n. Reference list of indicator species for the EUNIS 3 ecotope 'Shallow to moderate deep muddy substratum'.

EUNIS ecotope: Shallow muddy (Ondiep slibrijk)	Expected number of samples per year (2015+)		Data type of evaluation		R _i		IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning			
	Boxcore (0,078 m ²)	Dredge (15 m ²)	Hit rate (spatial)	Densities (number/m ²)	Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (15 m ²); densities (n/m ²)	General quality	A	B	C	D			E	F	G	H
Indicator Species																	
<i>Abra alba</i>		14		1		152,544	1	1	1	1	1	1	0,5	1			1
<i>Angulus fabula</i>		14		1		90,944	1	1		0,5	0,3	1	0,5				
<i>Donax vittatus</i>		14		1		0,598	1	1		0,5	0,3	1			1		
<i>Lanice conchilega</i>	3			1	248,700		1	0,5	1	0,5	0,1	1		1	1	1	1
<i>Liocarcinus holsatus</i>		14		1		5,521	1			1	1	1			1		
<i>Macoma balthica</i>		14		1		1793,180	1			0,5	1	1	1	1	1		
<i>Magelona johnstoni</i> + <i>M. filiformis</i>	3			1	520,000		1			0,25	0,3	1			1		1
<i>Nephtys hombergii</i>	3			1	76,000		1	1	1	0,5	0,5	1					
<i>Ophiura ophiura</i>		14		1		43,189	1	0,5	1	0,5	0,5	1			1		
<i>Owenia fusiformis</i>	3			1	60,000		1		1	0,25	0,5	1			1	1	1
<i>Spiophanes bombyx</i>	3			1	658,400		1			0,25	0,2	1					1
<i>Spisula subtruncata</i>		14		1		45,568	1	1		0,75	0,3	1	0,5	1	1		1
Number of species (S)	5	7					12	7	5	12	12	12	4	9	2		6

The specific evaluation to identify the possible impact of changes at the national level (F.) by taking characteristic species into account, and the specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account do not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Ophiura texturata* = *Ophiura ophiura*; *Magelona papillicornis* = *M. johnstoni* + *M. filiformis* (as *M. papillicornis* has never been present in the North Sea).

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Appendix 1o. Reference list of indicator species for the Habitat Directive habitat H1110c 'Sandbanks slightly covered by sea water all time' – Offshore subtype.

Natura2000 habitat H1110c	Expected number of samples per year (2015+)		Data type of evaluation		R _i	IV for Pressure				IV for recovery		National importance	IV for Ecological Functioning				Habitat Directive
Indicator Species	Boxcore (0,078 m ²)	Dredge (20m ²)	Hit rate (spatial)	Densities (number/m ²)	Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (20 m ²); densities (n/m ²)	General quality	A	B	C	D	E	F	G	H	I	J
<i>Acrocnida brachiata</i>	24			1	160,920		1			0,5	0,4	1	1	1		1	1
<i>Alcyonium digitatum</i>		15		1		0,006	1	1		1	1	0,5	1				1
<i>Angulus fabula</i>	24			1	331,013		1	1		0,5	0,3	1					1
<i>Aphrodita aculeata</i>		15		1		0,420	1	0,5	0,5	0,75	1	0,1	1				1
<i>Arctica islandica</i>		15		1		0,602	1	1	0,5	1	1	0,1					1
<i>Astropecten irregularis</i>		15		1		1,195	1	0,5		1	0,5	0,1	0,5				1
<i>Bathyporeia elegans</i>	24			1	490,375		1		1	0,25	0,1	1		1		1	1
<i>Bathyporeia guilliamsoniana</i>	24			1	100,585		1		1	0,25	0,1	1		1		1	1
<i>Buccinum undatum</i>		15		1		0,184	1	1	1	1	1		1				1
<i>Corystes cassivelaunus</i>		15		1		1,595	1	1	1	1	1	1		1		1	1
<i>Echinocyamus pusillus</i>	24			1	36,863		1		1	0,25	1	1					1
<i>Ensis ensis</i>		15		1		5,332	1	1		1	1	0,1	1	1			1
<i>Ensis siliqua</i>		15		1		1,144	1	1		1	1	0,1	0,5	1			
<i>Euspira pulchella</i>	24			1	57,688		1	0,5		0,5	0,3	1		1			1
<i>Gari fervensis</i>		15		1		1,184	1	1		0,75	1	0,5	1				1
<i>Goniada maculata</i>	24			1	92,950		1				0,1	1		1			1
<i>Iphinoe trispinosa</i>	24			1	18,300		1			0,25	0,1	1					
<i>Kurtiella bidentata</i>	24			1	1287,500		1										1
<i>Lanice conchilega</i>	24			1	474,040		1	0,5	1	0,5	0,1	1		1	1	1	1
<i>Liocarcinus holsatus</i>		15		1		0,175	1			1	1	1		1			1
<i>Luidia sarsii</i>		15		1		0,040	1						1				1
<i>Magelona johnstoni</i> + <i>M. filiformis</i>	24			1	775,638		1			0,25	0,3	1		1		1	1
<i>Nephtys assimilis</i>	24			1	12,813		1	0,5	1	0,5	0,6	1	1				
<i>Nephtys cirrosa</i>	24			1	43,263		1	0,5	1	0,5	0,5	1					1
<i>Nephtys hombergii</i>	24			1	181,420		1	0,5	1	0,5	0,5	1					1
<i>Neptunea antiqua</i>		15		1		0,010	1						1				1
<i>Ophiothrix fragilis</i>		15		1		0,075	1						1				1
<i>Ophiura ophiura</i>		15		1		0,166	1	1	1	0,5	0,5	1		1			1
<i>Owenia fusiformis</i>	24			1	73,713		1		1	0,25	0,5	1		1	1	1	
<i>Pagurus bernhardus</i>		15		1		0,728	1	0,5		1	1	1					1
<i>Psammechinus miliaris</i>		15		1		0,423	1	0,5	1	0,75	1	1	1				
<i>Sigalion mathildae</i>	24			1	52,888		1	0,5		0,5	0,4	1	1				1
<i>Spiophanes bombyx</i>	24			1	3730,780		1			0,25	0,2	1				1	1
<i>Urothoe poseidonis</i>	24			1	125,000		1			0,25	0,1	1		0,5			1
Number of species (S)	18	16					34	19	14	29	30	29	14	14	2	8	29

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Ophiura texturata* = *Ophiura ophiura*; *Amphiura brachiata* = *Acrocnida brachiata*; *Mysella bidentata* = *Kurtiella bidentata*; *Eupagurus bernhardus* = *Pagurus bernhardus*; *Magelona papillicornis* = *M. johnstoni* + *M. filiformis* (as *M. papillicornis* has never been present in the North Sea).

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Appendix 1p. Reference list of indicator species for the Habitat Directive habitat H1110b 'Sandbanks slightly covered by sea water all time' – Coastal subtype.

Natura2000 habitat H1110b	Expected number of samples per year (2015+)		Data type of evaluation	R_i		IV for Pressure					IV for recovery	National importance	IV for Ecological Functioning			Habitat Directive
	Boxcore (0,078 m ²)	Dredge (15 m ²)				General quality	A	B	C	D			G	H	I	
Indicator Species			Hit rate (spatial)	Densities (number/m ²)	Boxcorer (0,078 m ²); hit rate (per core) or densities (n/m ²)	Dredge (15 m ²); densities (n/m ²)										
<i>Abra alba</i>		188		1		29,166	1	1	1	1	1	0,5	1		1	1
<i>Angulus fabula</i>	40			1	306,860		1	1		0,5	0,3	1	1			1
<i>Bathyporeia elegans</i>	40			1	153,632		1		1	0,25	0,1	1	1		1	1
<i>Buccinum undatum</i>		188		1		0,011	1	1	1	1	1					1
<i>Donax vittatus</i>		188		1		1,797	1	1		0,5	0,3	1	1			1
<i>Echinocardium cordatum</i>	40			1	60,300		1		1	0,75	1	1	1		1	1
<i>Euspira pulchella</i>	40			1	28,604		1			0,5	0,3	1	1			1
<i>Lanice conchilega</i>	40			1	1989,266		1	0,5	1	0,5	0,1	1	1	1	1	1
<i>Liocarcinus holsatus</i>		188		1		0,902	1			1	1	1	1			1
<i>Lutraria lutraria</i>		188		1		0,944	1	0,5		1	0,5	1	1			
<i>Macoma balthica</i>		188		1		65,978	1			0,5	1	1	1	1		1
<i>Mactra stultorum</i>		188		1		0,026	1	1		0,75	0,6	1	1			1
<i>Magelona johnstoni</i> + <i>M. filiformis</i>	40			1	3166,468		1			0,25	0,3	1	1		1	1
<i>Nephtys cirrosa</i>	40			1	137,064		1	1	1	0,5	0,5	1	1			1
<i>Nephtys hombergii</i>	40			1	107,736		1	1	1	0,5	0,5	1	1			1
<i>Ophiura ophiura</i>		188		1		10,473	1	0,5	1	0,5	0,5	1	0,5	1		1
<i>Owenia fusiformis</i>	40			1	73,620		1		1	0,25	0,5	1	1	1	1	
<i>Pagurus bernhardus</i>		188		1		0,705	1	0,5		1	1	1	1			1
<i>Pontocrates altamarinus</i>	40			1	51,096		1	0,5		0,25	0,1	1	1			1
<i>Spiophanes bombyx</i>	40			1	1911,812		1			0,25	0,2	1	1		1	1
<i>Spisula subtruncata</i>		188		1		87,455	1	1		0,75	0,3	1	0,5	1	1	1
<i>Urothoe poseidonis</i>	40			1	770,988		1			0,25	0,1	1	0,5			1
Number of species (S)	12	10					22	13	9	22	22	21	5	14	2	20

The specific evaluation to indicate changes in habitat structure (H.) by taking species creating permanent structures into account does not meet the criterion of a minimum of 5 indicator species in the analyses.

Tellina fabula = *Angulus fabula*; *Fabulina fabula* = *Angulus fabula*; *Euspira poliana* = *Euspira pulchella*; *Lunatia poliana* = *Euspira pulchella*; *Euspira nitida* = *Euspira pulchella*; *Ophiura texturata* = *Ophiura ophiura*; *Eupagurus bernhardus* = *Pagurus bernhardus*; *Magelona papillicornis* = *M. johnstoni* + *M. filiformis* (as *M. papillicornis* has never been present in the North Sea).

Appendix 2. 'BISI Assessment Tool' worksheets with detailed methodology for each area to be evaluated.

Worksheets contain the complete detailed methodology for evaluation based on BISI including the creation of the references and a first calculation of the BISI values for the situation in 2015 (T0). Worksheets can be used in future evaluations by replacing the values for 2015 with the relevant values of another year to be evaluated. The spreadsheet is added as a separate file (BISI Assessment Tool v260917.xlsx).

Appendix 3. Overview of agendas Workshops / Expert Meetings 'Benthos Indicator Development', from November 7, 2016 and February 16, 2017 with participants lists.

agenda

Omschrijving	Workshop benthos indicator
Voorzitter	Dennis van Schaardenburg
Vergaderdatum en -tijd	7 november 2016, 10.00 uur – 15.00 uur
Locatie	ntb

Vergaderpunten

1. Opening / mededelingen vooraf
2. Inleidende presentaties: setting the stage
 - a. Achtergrond benthos indicator en het monitoringsprogramma (Suzanne Stuijfzand) (10 min)
 - b. Monitoringsprogramma: gemaakte keuzes (Sander Wijnhoven) (30 min)
 - c. Intermediate Assessment Oskar: Common Indicator BH2 Multi-metric index (Willem van Loon a.h.v. *bijlage 1*) (15 min)
 - d. Habitatrichtlijn/beheerplannen N2000 (15 min)
 - e. Geoweb Viewer en benodigde data (Serge Rotteveel) (15 min)
 - f. Inleiding naar middagdeel (10 min)
3. Lunch (12.30-13.10)
4. Middagdeel
 - a. Plan van Aanpak: denkrichting voor benthos indicator en de stappen die je kunt nemen (Oscar Bos/Sander Wijnhoven) (40 min) – zie *bijlage 2*
 - b. Discussie (40 min), inzoomend op
 - i. Input experts op de stappen
 - ii. Overige zaken van experts
 - c. Conclusies (15 min)
 - d. Afronding

Participants: Anne-Marie Svoboda (EZ), Dennis van Schaardenburg (WVL), Suzanne Stuijfzand (WVL), Serge Rotteveel (WVL), Willem van Loon (WVL), Annemiek Adams (EZ), Peter Heslenfeld (ZD), Ad Stolk (ZD), Johan Craeymeersch (WUR), Karin van der Reijden (RUG), Edwin Verduin (Eurofins), Vincent Escaravage (NIOZ), Oscar Bos (WUR), Sander Wijnhoven (Ecoauthor).

Participating tot the discussion via email outside the workshop: Tobias van Kooten (WUR).

agenda

Omschrijving	Workshop benthos indicator
Voorzitter	Dennis van Schaardenburg
Vergaderdatum en -tijd	16 februari 2017, 9.30 uur – 14.00 uur
Locatie	BCN, Catharijnesingel 48, Utrecht

Vergaderpunten

1. Opening / mededelingen vooraf
2. Benthis-Beauchard methodiek (Ger Jan Piet)
3. Methodiek uit rapport (Sander Wijnhoven)
 - a. Inleiding
 - b. Vergelijking 2 methodieken
 - c. Ingezonden reacties - bijlage 1
4. Lunch (12.00-12.45)
5. Stand van zaken datamanagement Geoweb (Serge Rotteveel)
6. Discussie nav punt 3
7. Conclusies en afronding

Participants: Anne-Marie Svoboda (EZ), Dennis van Schaardenburg (WVL), Suzanne Stuijzand (WVL), Serge Rotteveel (WVL), Willem van Loon (WVL), Maarten Platteeuw (WVL), Hans de Ruiter (WVL), Peter Heslenfeld (ZD), Joël Cuperus (CIV), Edwin Verduin (Eurofins), Vincent Escaravage (NIOZ), Tobias van Kooten (WUR), GerJan Piet (WUR), Sander Wijnhoven (Ecoauthor).

Additional meeting to discuss results of the workshop with: Johan Craeymeersch (WUR).