Protocol Benthic Indicator Species Index (BISI):

Protocol BISI for generic application (BISI v2). Version v311219 as an update of v181218.

– Wijnhoven (2019)

This protocol describes the Benthic Indicator Species Index (BISI). The current version is the protocol for generic application: BISI v2 and comes with detailed Assessment tools:

In case of application in the Dutch context making use of multiple sampling techniques as part of dedicated monitoring programmes:

- *Wijnhoven (2019a). Assessment tool: 'Benthic Indicator Species Index (BISI)': Application of BISI v2 in the Dutch North Sea with consolidation of earlier identified references. v311219.*
- *Wijnhoven (2019b). Assessment tool 'Benthic Indicator Species Index (BISI)': Application of BISI v2 for marine Habitat Directive habitat types of the Dutch 'Delta-waters', the Wadden Sea and the coastal zone of the North Sea. v070120.*

In case of an international context developed for application based on (approximately) 0.1 m² grab or core samples:

- *Wijnhoven (2019c). Assessment tool: 'Benthic Indicator Species Index (BISI)': Application of BISI v2 in soft sediment habitats of OSPAR region II (Greater North Sea region). v311219.*

The BISI was initially developed as the national benthos indicator for evaluation of the quality status and sea floor integrity of Dutch North Sea areas, The focus with BISI v1 used to be on evaluation of the quality status and developments of areas of specific ecological value (ASEV) indicative for the quality status of the Dutch EEZ, and additional sampling to extrapolate findings to national level and for evaluation of effectivity of specific measures. Area specific methodologies (BISIs) were developed, from which the methodologies for 6 soft sediment broad habitat types were derived, (BISI v1 dated 26-09-17 is still in use for application and reporting for the Marine Strategy Framework Directive (MSFD) and delivers to the Habitat Directive (HD) Article 17 reporting and Natura 2000 fishery measures evaluations in the Netherlands). The current protocol is an update of BISI v2, developed for generic application in a variety of systems and the international context, by constructing BISIs at the level of ecotopes (broad habitat types). From these, area specific BISIs are composed based on ecotope surface ratio. BISI v2 is developed and in use for evaluation of the quality status of HD marine habitat types in the Netherlands and suggested being part of OSPAR indicator BH1 (Typical Species Composition) with regards to the Greater North Sea region in particular (see updated Draft CEMP BH1, 2019).

- *Draft CEMP BH1 (2019). OSPAR- Biodiversity Indicators Candidate Indicator Typical Species Composition (BH1); DRAFT Generic guidelines for Coordinated Environmental Monitoring Programme (CEMP).*

BISI v2 is different from v1[1] *as with v2:*

- *ecotopes form the basis for the derivation of the index;*
- *(standardized) rules for indicator species selection (and the construction of BISIs for other ecotopes/regions) are now part of the methodology;*
- *natural logarithms (ln) instead of* ^{10}*log are used now to overcome skewness of the results;*
- *the use of reference areas (in line with the draft CEMP for BH1) has a more prominent role in the derivation of reference values now;*
- *some corrections on how to calculate pooled standard deviations associated with BISI-scores are implemented,*

This allows BISI development and application in basically any area or region with sufficient data availability.

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[1] BISI v1 results and recent historic trends indicating directions of changes are still valid (significance levels at T0 might however deviate). Former results for the T0 of the Dutch North Sea (and recent historic trends) can relatively easy be recalculated into the scores according to BISI v2 maintaining the earlier defined indices (indicator species lists and reference levels). An update of the indices is not necessary for evaluation of quality status and developments of individual areas; it might only be desirable for a better comparison of the differences in relative quality status between areas, and comparison with areas outside the Dutch North Sea.
Background information on specifically the development of BISI v1 is described in:
- Wijnhoven, S. & Bos, O.G. (2017). Benthic Indicator Species Index (BISI): Development process and description of the National Benthos Indicator North Sea including a protocol for application. Ecoauthor Report Series 2017 - 02, Heinkenszand, the Netherlands.

Results of application of the BISI are presented in:
- Wijnhoven, S. (2018). T0 evaluation of the quality status of the Dutch Exclusive Economic Zone based on the Benthic Indicator Species Index (BISI). Quality status and – developments of benthic habitats and MSFD areas of the Dutch North Sea in and prior to 2015. Report Ecoauthor & Wageningen Marine Research. Ecoauthor Report Series 2018 - 01, Heinkenszand, the Netherlands.

This includes the factsheet: 'Benthic Indicator Species Index (BISI)': D6C3/5, as prepared for the Dutch Action plan Marine Strategy (part 1) 2018-2020:
- Min IenW, Min LNV (2018). Mariene Strategie (deel 1). Huidige milieutoestand, goede milieutoestand, milieudoelen en indicatoren 2018-2024, Hoofddocument. Een uitgave van Ministerie van Infrastructuur en Waterstaat en Ministerie van Landbouw, Natuur en Voedselkwaliteit, februari 2018, wvl0118tp312 (in Dutch).

This protocol should be cited as:

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The protocol is based on the structure of the ICES WGBIODIV template for Indicator factsheets (version 0.1) which is developed by O. G. Bos.

Index

Benthic Indicator Species Index (BISI)

$$BISI = \exp((1/S)^* \Sigma \ln(IV_i{}^*(O_i/R_i)))$$

BISI = Benthic Indicator Species Index; S = Number of indicator species included; IV_i = Species specific Indicator Value calculated as species specific standard indicator value iv_i (value between 0-1) divided by average indicator value iv_{avg}; O_i = Observed occurrence species i (either presence/absence ratio, density or biomass); R_i = Reference occurrence species i (presence/absence ratio, density or biomass under reference conditions). ('exp' is similar to putting e to the power of the formula as indicated, which equals the inverse natural logarithm, as a back-transformation of the natural logarithm (ln) taken from the occurrence-to-reference ratios).

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2 Contributors

Contributors to the current version: P.H. van Avesaath (AMAECON) assisted in developing the methodology for BISI v2. P.H. van Avesaath and L. Soldaat, M. Poot and R. Beij (Statistics Netherlands) gave valuable comments on the methodology presented here. Ideas and development of the BISI are presented and discussed during project meetings (BISI development under different contracts and for different applications); special thanks to clients and contact persons involved; A.-M. Svoboda (Dutch Ministry of Agriculture, Nature and Food Quality; Min LNV, formerly Min EZ), S. Stuijfzand (Ministry of Infrastructure and Water Management; Min IenW, formerly Min IenM) and J.B.F. Vonk (Min LNV). Special thanks to O.G. Bos (Wageningen Marine Research) being the editor of protocol v1 and to all experts and potential users that commented on earlier versions, applications and/or were involved in discussions: A.-M. Svoboda, A. Adams (Min LNV), S. Ciarelli, J. Cuperus, P. Heslenfeld, R. Hoeksema, W. van Loon, M. Platteeuw, J. Postema, H. Ruiter, S. Rotteveel, D. van Schaardenburg, J. Staeb, A. Stolk, S. Stuijfzand, G. Vossebelt, M. van Woensel (Min IenW), J. Craeymeersch, G.J. Piet, T. van Kooten (Wageningen Marine Research), Beauchard, O., Escaravage, V. (Netherlands Institute for Sea Research), E. Verduin (Eurofins), A. van Strien (Statistics Netherlands). The BISI is presented and discussed within the OSPAR Benthic Habitats Expert Group (OBHEG) and presented within the EU MSFD Technical Group Seabed.

3 Key message

The Benthic Indicator Species Index (BISI) uses benthos data to evaluate habitat quality, sea floor integrity and ecological functioning. BISI compares temporal patterns (densities or absence/presence) of combinations of specific in-indicator species with predefined reference levels (BISI internal reference) of these species that represent a good status. The selection of indicator species and derivation of reference levels is part of the methodology and takes place at the level of (high aggregation) ecotopes (e.g. MSFD broad habitat types (EUSeaMap, 2019) or other ecotope classifications with clearly to distinguish benthic communities). BISI can be used at different spatial scales by combining ecotope-specific results on basis of surface ratios; e.g. (sub)regional scale, national scale, Natura 2000 or specific management areas, etc.). BISI can be used to evaluate current quality status, as well as trends in quality status, e.g. to determine the effect of management measures in protected areas.

Indicator species are selected to a standardized derivation scheme per ecotope on basis of being characteristic, or being indicative for at least one of the dominant disturbances, or being characterized by a combination of biological traits. Selected species should have opportunities to be present or return as well. Reference values are preferably obtained from suitable reference areas. When reliable reference data lack, (BISI) internal reference values are derived from current and maximum observed occurrences and natural variation. Therefore a standardized decision scheme, taking recent historic data availability and current monitoring efforts, possible availability of reference (as indicated) or alternative areas, and suitability of monitoring techniques or possible alternative techniques, is used as guideline.

The BISI provides an evaluation of the general quality status of an ecotope (habitat) or aggregated ecotope area and several specific evaluations to identify possible causes and effects of the observed quality status (and their importance), based on subsets of indicator species. Specifically developed for the benthic quality status in marine waters based on benthos; application in other systems and types of habitats and inclusion of other types of biota as indicators is possible.

4 Definitions

Area specific index – Index designed/compiled for a specific area to be evaluated. Here used as the area specific BISI; indicating that each area of evaluation has its BISI, where indicator species composition with their reference occurrences depends on the constitution of the area. With BISI v2 the compilation of the area specific reference is more standardized, build on ecotope presence (surface area based) with each ecotope its commonly used standard reference.

ASEVs	- Areas with special ecological values. Designated areas in Dutch North Sea that include all Natura 2000 areas (and therefore the areas of the Habitat Directive), often with specific management measures; focal areas of the Dutch North Sea benthos monitoring programme.
BH1	- OSPAR Biodiversity Indicators - Benthic Habitat indicator 1: Candidate Indicator Typical Species Composition (OSPAR, 2018) of which the current draft proposal includes the BISI integrated as part of Typical Species Composition (OSPAR, 2019).
BISI	- Benthic Indicator Species Index. Benthos-based indicator for quality status assessment and evaluation of quality developments of benthic habitats and sea floor integrity in particular.
BISI-score	- Score (also indicated as BISI-value) that reflects the benthic habitat quality status. In practice in case of BISI v2 the score deviates between 0.01 and 100. A value approaching the lower limit means very poor quality; a value around 1 equals a relative good quality status similar to the internal reference (potentially within reach on the mid-long term for areas under pressure when dominant pressure levels are significantly decreased). Taking uncertainty and realistic monitoring efforts into account; a BISI-value of 0.5 might be a suitable threshold value with regards to the MSFD for the time being.
BISI v1	- Original version of the Benthic Indicator Species Index, specifically developed for evaluation of areas and habitats of the Dutch North Sea. V1 is especially deviating from BISI v2 in the way references are constructed area (ASEV) specific. In v2 an adjustment of the transformation and back-transformation has taken place so that results in analyses of quality developments (trends) are less skewed[2].
BISI v2	- Updated Benthic Indicator Species Index, especially deviating from BISI v1 in the way that references are constructed ecotope specific. A reference for an area to be evaluated is built surface area based from the standard references of the present ecotopes. In v2 the transformation and back-transformation leading to more skewed results in analyses of quality

[2] Transformation in v1 was according to ^{10}log (instead of a natural logarithm in v2) while back-transformation consisted of the inverse natural logarithm. Results of the two (v1 and v2), point in the same direction, but there is a difference in the steepness of trends.

developments (trends) is adjusted. Other deviations are a further standardization of indicator species selection, reference level derivation and corrections in the methodology of calculating pooled standard deviations associated with the BISI.

Closed areas - A term used in management of the Dutch North Sea, indicating that there are certain fisheries restriction in the areas of concern. In general those areas are not complete closed for all human activities or potential human disturbances; in many cases only certain sea floor disturbing fisheries (i.e. beam trawling) is banned.

Ecological disturbance – Disturbance of habitat and benthic communities in particular due to sensitivity of species for toxic substances, pollutants, elevated nutrient levels (and likely also hypoxia or temperature elevations). Indicated pressures often have an impact on the same species in about similar amounts and often co-occur to some extent.

Ecotope - Relatively homogeneous, spatially explicit landscape unit described by abiotic conditions reflecting a certain biotic constitution and development. Although BISI can potentially function based on any ecotope classification system, preferably widely accepted classifications like EUNIS (e.g. MSFD broad habitat types) and ZES.1 are suggested.

EUNIS - European Nature Information System (www.eunis.eea.europa.eu/index.jsp). Here specifically the EUNIS habitat classification system is adapted, where the EUNIS classification at current level 2 (indicated as MSFD broad habitat types) or aggregations of those are suggested for application of the BISI at the European regional seas level.

HD habitat types – Characteristic habitat types that are protected under the Habitats Directive (HD) in those areas specifically designated as Habitats Directive areas (part of the EU Natura 2000 network).

Hit rate - Alternate expression for 'occurrence' or spatial distribution of an indicator species in an area of evaluation.

Indicator Value – Indicated with IV_i, the indicator value is the species specific indicator value iv_i with per definition a value between 0-1, divided by the average indicator value (iv_{avg}) of all indicator species in the specific evaluation ($IV_i=iv_i/iv_{avg}$).

Internal reference or Realistic reference – A reference occurrence, abundance or biomass for an indicator species (or a combined BISI-score based on a set of indicator species) reflecting a quality status that can be reached on the mid-long term when dominant pressures are significantly reduced to a level of minimal impact. The internal references used in BISI are derivate from maximum potential observations taking natural fluctuations into account, when no data from reference areas are available. With the current indicator the reference BISI-score per definition equals 1; taking uncertainty and realistic monitoring efforts into account, a value of 0.5 might be a practicable threshold value for good quality.

MSFD - Marine Strategy Framework Directive.

MSFD broad habitats – Habitat types specifically distinguished in the current EUNIS classifications (EUSeaMap, 2019), suggested by the EU as the working and evaluation units with regards to the MSFD (reporting).

Occurrence - Presence/absence of species at a monitoring location (in a standardized benthos sample) to reflect the spatial distribution of an indicator species or the presence of indicator species in densities of biomass.

Open areas - A term used in the monitoring and evaluation of Dutch North Sea areas. Monitoring sites are selected in open areas (areas with no specific restrictions) that are comparable in habitat or ecotope constitution or in constitution of the expected benthic communities, with monitoring sites situated in 'closed areas'. Herewith evaluation of efficiency of management measures (closing areas for certain sea floor disturbing fisheries) is according to a BACI design.

OSPAR - OSPAR is the mechanism by which 15 Governments and the EU cooperate to protect the marine environment of the North-East Atlantic, named after the original Oslo and Paris Conventions.

Pristine - Undisturbed condition reflecting the 'natural' situation of before substantial anthropogenic influence.

Reference - A standard value to compare with. Here a standard value of occurrence, abundance or biomass for an indicator species (or the combination of species in a BISI-score) to compare observation data with. Here a pristine and an internal (realistic) reference are distinguished.

Smart species – 'Smart species' is a term introduced in Wijnhoven et al. (2013) for potential indicator species (sensitive for specific pressures or representative for certain ecological functions of the habitat of concern) for which differences in spatial occurrence can be detected with a realistic monitoring effort using common and/or available monitoring techniques. In the current methodology this realistic effort is set to 60 samples as a maximum number allowing detecting at least 50% differences in species spatial occurrence with a power of 80% at a significance level of 0.05.

Species specific indicator value – Indicated with iv_i, the species specific indicator value is a valuation of a characteristic of a potential indicator species deviating between 0-1 with 0 = no indicator value at all (not included in a the representative specific evaluation) and 1 = a very good indicator for the specific evaluation.

Threshold value – A value that should be reached to indicate a good quality status; at the moment there are no established threshold values yet for amongst others (most) MSFD and OSPAR indicators. In case of use of the BISI for quality evaluation, a threshold of BISI=0.5 is suggested for good quality status, with the ultimate goal to reach a BISI of at least 1.

Typical species – Term originally from the Habitats Directive; typical species are selected by countries on basis of their presence being considered indicative for either or both a good biotic or abiotic structure and function of the habitat type of concern (e.g. Shaw & Wind, 1997).

- Within the frame of OSPAR; the typical species composition (BH1) is suggested as the indicator to evaluate the quality status of benthic habitats. Typical species are here those species clearly distinguishing between reference - and impacted benthic communities (as identified by species dissimilarity analyses). It is suggested to combine the original typical species composition indicator with the BISI as BH1 (OSPAR, 2019), as such that the first can be used in case of clear uni-pressure situations in the presence of reference areas, whereas the BISI could be used in case of multi-pressure situations and/or the lack of reference areas and to identify the pressures responsible for the observed quality status. Results from the Typical Species Composition indicator applied to suitable uni-pressure situations can be used to define the internal reference of the BISI.

Typical Species Composition – OSPAR benthic habitat indicator BH1 - originally a methodology to select a set of indicator species from a comparison of occurrence of species in reference compared to known single pressure impacted areas and test the occurrence of those species in other areas with the same habitat type (Spanish proposal Candidate Indicator BH1; OSPAR, 2018). At present there is a draft proposal in which the BISI is integrated as being part of the proposed BH1 'Typical Species Composition' indicator (OSPAR, 2019) as an alternative methodology for evaluation is case references areas lack or in case of less defined possible multiple pressure situation, at which however the original Typical Species Composition methodology can provide the input for the construction of BISIs for other areas.

ZES.1 - 'Zoute wateren Ecotopenstelsel' (Bouma et al., 2005: Marine environments ecotope classification system). Adapted as the basis for ecotope classification in BISI for 'transitional' (marine and estuarine waters on the transition from land to sea) larger waterbodies in the Netherlands.

5 Indicator metadata

5.1 Ecosystem component

Marine benthos: Endofauna and sessile epifauna species of which populations can be monitored with techniques giving a representative inventory like corers, grabs, dredges, trawl tows and video recordings.

5.2 MSFD Descriptor

D6 Seafloor integrity (C3 and C5 according to the Dutch Marine Strategy: Respectively 'the spatial extent and quality of habitats potentially impacted by changes in biotic and abiotic structures and functions' and 'changes in size, condition and distribution of populations of benthic macrofauna species') and D1 Biological diversity (C5 of the (benthic) habitat for species has the necessary extent and condition to support the different stages in the life history of the species).

5.3 Status

BISI is an indicator that has been developed in the Netherlands in 2016/2017 for the Ministry of Economic Affairs. It has been used to evaluate the quality status and the recent historic quality developments in and prior to 2015 for the MSFD (Wijnhoven, 2018), and is part of the Dutch Marine Strategy (Min IenW & Min LNV, 2018). The indicator has no status within OSPAR yet, but within OBHEG it has been agreed that the BISI will be part of the test - and comparison activities towards common OSPAR indicators and the Benthic Habitat indicator BH1 in particular, in which the Netherlands and Spain are co-leading. The current protocol (BISI v2) is suggested for this and a draft proposal for integration in BH1 Typical Species Composition is available now (OSPAR, 2019).

5.4 Indicator type

State indicator.

6 Indicator description

6.1 Indicator goals/objectives

The Benthic Indicator Species Index (BISI) aims to evaluate the quality status, quality status developments and quality status differences for defined spatial areas/ecotopes.

In addition, the BISI aims to evaluate the importance of different potential pressures and the effect of the potentially decreased quality on ecosystem functions (See amongst others Tables 6.3.3.a and 6.3.3.b). Overall quality indices for each of the identified areas to be evaluated are combined with specific quality indices that are based on weighted subsets of indicator species with specific characteristics and traits. Indices (BISIs) are developed at the level of ecotopes (broad habitat types) and combined surface ratio based for areas to be evaluated. Therefore the BISI will also identify possible changes or developments in habitat constitution (either as a result of natural development or due to anthropogenic reasons) in case of sufficient monitoring. The BISI intends to be a well-documented, reproducible and efficient assessment method that includes a protocol and assessment tools.

BISI v1 is specifically geared for evaluation of areas of the Dutch North Sea. With BISI v2, the approach has been generalized, making it applicable in areas across the world so that it will be possible for every benthic expert to construct indices (BISIs) for their own area of interest, if (recent historic - or reference area - and current -) data availability is sufficient.

6.2 Theoretical background

Macrobenthos, macrobenthic communities and benthos indicator species in particular are expected to reflect the quality status of the (local) environment, and especially that of the benthic habitats (sea floor integrity) (e.g. Ysebaert et al., 2002; Reiss et al., 2015; Elliott et al., 2018). Most benthic species have a strong relation with the constitution of the sea floor, are typically related to the prevailing abiotic condition (that can basically be described with a limited number of abiotic parameters) and the quality status of that habitat determined by the (former) presence (or absence) of pressures of different kinds and/or severity (e.g. Hiscock et al., 2004; Dutertre et al., 2013). Additionally the biotic conditions are of importance, where the presence (or absence) of certain species or communities can provide improved habitat quality, amongst others via influencing local abiotic conditions, possibly accelerating the settlement and/or natural succession of benthic communities. Natural development of good quality benthic habitats includes habitat rejuvenation and the presence of a variety of different habitat elements in various developmental stages. This indicates that the presence of typical habitat related species[3] in their potential abundances reflect the quality status suggesting that there are opportunities for those species to arrive/return. Hampered opportunities for those typical habitat related species to return are more an indication of former presence of large disturbance levels at larger scale resulting in the lack of source populations and/or poor connectivity. Species as indicated are potential indicator species, especially when they are sensitive for specific pressures. To be suitable as an indicator species, also natural fluctuations in occurrences should be relatively small compared to fluctuations under the influence of a specific pressure, and monitoring of species natural occurrence should be possible using conventional observation techniques and relatively low monitoring efforts.

With BISI the combined occurrence of indicator species of the general quality status related to specific habitats are evaluated. Additionally, further differentiation in specific evaluations solely based on indicator species specifically sensitive and/or indicative for specific pressures and/or functions indicate the status of specific causes or effects of the observed quality status. Comparing the quality status based on BISI for different years allows evaluation of quality status developments and identification of possible thriving factors and or resulting effects on ecosystem level.

6.3 Description of the indicator

6.3.1 Benthic Indicator Species Index (BISI):

The occurrence (spatial occurrence as presence/absence data, abundances or biomass) of an (area - and/or habitat specific) selection of indicator species at a certain moment of evaluation is compared with defined reference values for that selection of indicator species. The methodology consists of the calculation of the weighted (species - and evaluation specific indicator values) geometric mean (i.e. ln-transformed) of observation-to-reference ratios. Testing occurs against a (fixed) reference compiled in a standardized way for ecotopes, and for other composite areas like areas with a certain protection status or importance or Habitat Directive habitat types (covering several ecotopes).

[3] Although Typical Species of the Habitats Directive are (also) selected as their presence is considered indicative for either or both a good biotic or abiotic structure and function of a habitat (Shaw & Wind, 1997), the list of potential indicator species considered here is more extensive. With BISI in the end not only species presence is evaluated, but optionally also differences in other occurrence data (e.g. spatial occurrence, densities, biomass) that allow quantification and statistical testing. The typical species composition methodology (OSPAR BH1: OSPAR, 2019) foresees in distinguishing the most sensitive indicator species per ecotope/habitat type comparing reference - with uni-pressure impacted communities. Those results can potentially be used for construction of the ecotope/habitat specific BISI (in case a BISI is not available yet).

References are compiled surface ratio based from the individual (most important) ecotope references in a standardized way.

The BISI evaluation tool was initially developed (as BISI v1) for evaluation of the Dutch North Sea including all benthos-based specific evaluations needed for MSFD reporting, effectivity of management regulations evaluations, and providing background information on causes of and functions affected by observed changes in the quality status, amongst others of importance towards Natura 2000 and Habitat Directive evaluations (Figure 6.3.1.a).

At present specific BISIs are developed for evaluation of areas with special ecological values (ASEVs of which several are also Natura 2000 areas) in the Dutch North Sea: i.e. Dogger Bank (Doggersbank), Cleaver Bank (Klaverbank), Central Oystergrounds (Centrale Oestergronden), Frisian Front (Friese Front), Brown Bank (Bruine Bank), North Sea Coastal Zone (Noordzeekustzone), 'Front Delta' (Voordelta), 'Plain of the Raan' (Vlakte van de Raan) on basis of BISI v1 making use of a dedicated monitoring programme consisting of boxcore and dredge samples (and Hamon grab and video specifically on the Cleaver Bank) (Wijnhoven & Bos, 2017). Based on the methodology for the mentioned (for the Dutch North Sea) representative areas, also BISIs were defined for six EUNIS level 2 ecotopes (broad habitat types); 'Offshore circalittoral coarse sediment', 'Offshore circalittoral sand', 'Offshore circalittoral mud', 'Circalittoral coarse sediment', 'Circalittoral sand', 'Circalittoral mud'. From these also BISIs for the Habitat Directive habitat (sub)types H1170 'Reefs', H1110b 'Submersed sandbanks in the Coastal zone', H1110c 'Offshore submersed sandbanks' were constructed. Efficiency of management regulations (fisheries restrictions in specific areas) are evaluated using the same BISIs as for related ASEVs based on a dedicated monitoring design (BACI approach). Now also an assessment tool according to the v2 formula to calculate a quality score (BISI) is available to put results into perspective with other applications, however with consolidation of earlier indicated reference levels at ASEV level (Wijnhoven, 2019a).

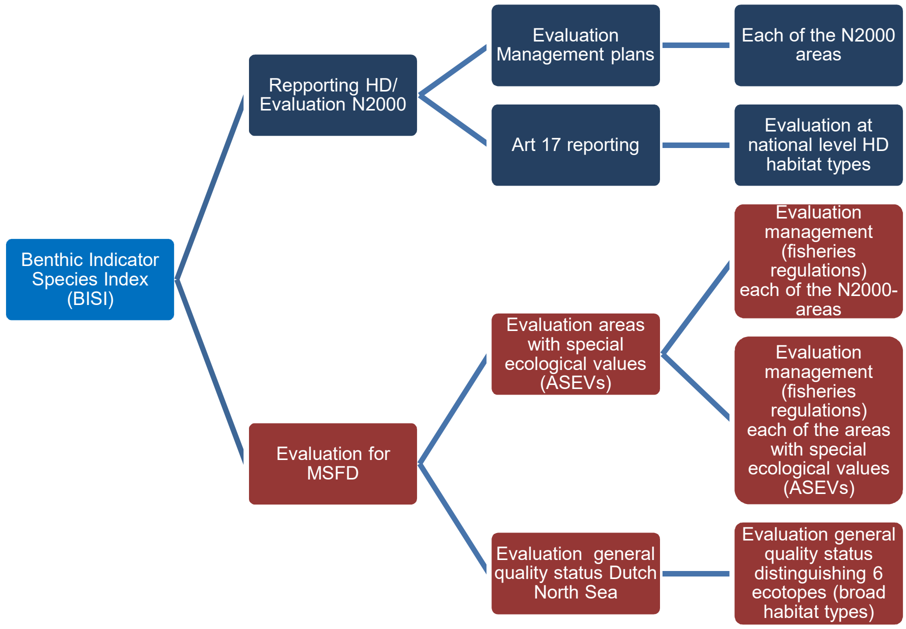


Figure 6.3.1.a Schematic overview of the different Dutch evaluations for which the Benthic Indicator Species Index (BISI) is developed as BISI v1.

According to BISI v2, BISIs are developed for a range of ecotopes related to HD habitat types specific for marine and estuarine waterbodies. Combined BISIs for respectively 5, 8, 3, 3 and 2 ecotopes (according to classification system ZES.1; Bouma et al., 2005) form the bases of the BISIs for respectively HD habitat (sub)types H1160 'Large shallow inlets and bays', H1130 'Estuaries', H1110a 'Sandbanks permanently flooded - subtype Tidal area', H1140a 'Intertidal mud flats and sandbanks - subtype Tidal area' and H1140b 'Intertidal mud flats and sandbanks - North Sea coastal zone' (Wijnhoven & Van Avesaath, 2019). BISIs are specifically elaborated in an assessment tool for those HD areas as present in the Dutch south-western delta, the Wadden Sea and the coastal zone of the North Sea (Wijnhoven, 2019b).

Additionally BISIs are in development for evaluation of the six earlier mentioned ecotopes (broad habitat types) as identified for the Dutch North Sea for application in the international context within the frame of OSPAR with regards to the Greater North Sea region (region II), based on (and suitable for) approximately 0.1 m^2 grab or core samples (methodology presented as Annex 8 of OSPAR CEMP BH1; OSPAR, 2019). Suggested assessment tool (Wijnhoven, 2019c) is available for application and testing in the international context.

6.3.2 Selection of ecotopes

With the current protocol, BISIs are basically developed at the level of ecotopes. Dependent of the aim of the evaluations, ecotopes can be either broad habitat types or very detailed defined habitats. Broad habitat types are typically used for broad and large scale applications, like for (sub)regional or national evaluations (e.g. with regards to MSFD, OSPAR, HD). Evaluation of detailed habitats might for instance be useful in case of evaluation of specific local disturbances or management measures often project based, in presence of intensive dedicated monitoring. Something in between could be application of the BISI specifically for evaluation of status of HD eco-elements or OSPAR protected and declining habitats, like certain faunal reefs and beds. It is of importance that identified ecotopes (or habitats) are clearly distinguishable (also) on basis of benthic communities under natural conditions where classification boundaries are (most) logic.

In case of specific attention for a certain habitat, BISIs can be developed for a single type of habitat. However, in case of application to larger areas with more abiotic variability, it is often needed to distinguish several ecotopes for which separate internal references (as used in BISI) are developed. Ultimately a combined internal reference is obtained surface ratio based. Larger scale ecotope classifications for benthic habitats are generally based on hydrodynamics, salinity, substrate, depth or duration of exposure in case of the intertidal zone or derivate/related aspects like photonic zone. At present the use of EUNIS classifications (e.g. broad habitat type related classification; EUSeaMap, 2019) is suggested for the international context; in the Netherlands, ZES.1 (Bouma et al., 2005) is suggested for WFD transitional (marine and estuarine) waters.

It has to be taken into account that distinguished ecotopes (and therefore derived/suitable internal references for the BISI) might show (sub)regional differences and/or waterbody related differences. A more detailed classification might provide a more accurate evaluation, however, might also need a comprehensive monitoring programme with sufficient representative monitoring in each of the ecotope types. A rule of thumb might be that a clearly distinguished ecotope is taken into consideration when it represents more than 10 % of the total area to be evaluated (certain less common ecotopes might however be judged as of significant importance to the overall quality status, in terms of biodiversity or ecological function and can be included in that case).

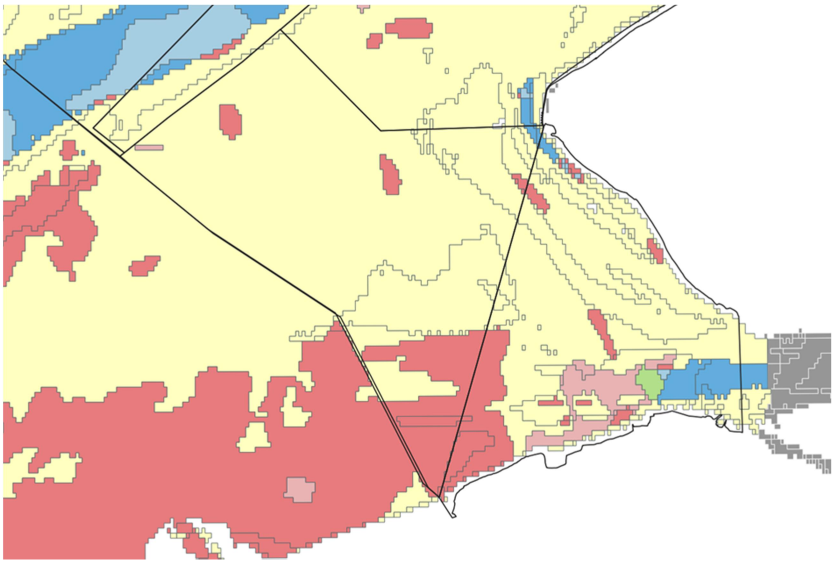


Figure 6.3.2.a Example of Natura 2000 area 'Vlakte van de Raan' (area in the middle) subdivided into ecotopes on basis of depth (shallow, deep) and sediment type (mud, sand, coarse sediment). Two ecotopes are distinguished on basis of which an area specific BISI is developed (shallow sand – 85%; shallow mud – 15%) In the Netherlands, the Vlakte van Raan is designated HD habitat subtype H1110b and evaluated as such. Also coarse sediment could potentially be present in this type of habitat but this is hardly the case here, so it is not taken into account for the evaluation of the 'Vlakte van de Raan'. A tiny part appears to be 'deep', but as this is atypical for H1110b (and actually unwanted for a good quality status), this is not taken into consideration in the reference. If a significant part of the area becomes deeper in the future (e.g. due to coastal works and changed hydrodynamics in the vicinity) this might be observed in the quality assessment in case of sufficient monitoring due to impact on the benthic communities; as the ecotope type is not included in the reference a decreasing BISI-score (quality status) is expected in such case. Indicated boundaries within distinguished ecotopes do indicate the difference between infralittoral and circalittoral area in this case (both indicated as 'shallow' now); not used in the current evaluations in the Netherlands. Possible changes in these sub ecotopes, although not specified in the reference, might be reflected in the observed quality status as well, as they likely lead to changes at the community level; after which it should be identified whether changes are of natural or anthropogenic origin.

6.3.3 Selection of indicator species

Potential indicator species are selected on basis of a few characteristics making use of evidence from scientific literature, occurrence in historic datasets and/or species characterization in standard catalogues/databases at the level of (high aggregation) ecotopes:

1) Relatedness to ecotopes: Certain species are characteristic or specific for certain habitats (common or abundant in specific ecotope whereas less abundant or absent in most other ecotopes) and are therefore potential indicators for changes in habitat constitution.
2) Indicative for one of the dominant disturbances: Expected potential dominant disturbances in the area of concern are identified (E.g. in the

Greater North Sea region these are generally 'seafloor disturbance' and 'ecological disturbance (basically impact of nutrients and/or pollutants)', but it might be disturbances like increased turbidity, hydrodynamics or inundation time due to human activities, or presence of exotic species, or temperature increase, or other disturbances in other cases).

3) Species traits/life histories: Benthic macrofaunal species can be characterized in terms of sensitivity, resistance (to pressures) and/or resilience (recovery after pressure) towards different pressures in their direct environment or habitat. This characterization is largely correlated to the species' life-history indicated by specific traits (size (a), longevity (b), frequency and number of recruits (c)) with relevance towards disturbance and recovery.

All potential indicator species are scored on above aspects on a scale from 0 (not the case at all) to 1 (entirely the case). Species scoring '1' for aspects 1 or 2, or at least a summed score of 1.5 for aspects 3a-c, are eligible for selection.

4) Sensitivity of species in terms of power: Presence in terms of densities and distributions (e.g. equally distributed or present in aggregations with sufficient chance of detection) under natural good quality conditions and catchability with the monitoring available and applied sampling techniques, is tested. Criterion is that it should be possible to detect at least 50% changes in the spatial occurrence (presence/absence) of species with a reliability of 80% (significance level of 0.05) in one of the preferred ecotopes (aspect 1 ≥0.5) for the species, to be selected as an indicator species for the general quality status.
5) Last additional criterion is that species should have the opportunity to return (is at least present somewhere in the vicinity).

As indicated, the selection of indicator species is based on evidence from literature. It is suggested to work towards a database of potential indicator species for BISI with indication of relevant indicator values, where possible adapt information from widely accepted sources like WoRMS (Marine Species Traits editorial board, 2018), BIOTIC (MarLIN, 2006) and AMBI (Borja et al., 2000). Indicator species lists (and reference values) are ecotope specific; for each new area to be evaluated, references can be constructed from earlier references when identical or comparable ecotopes have been part of evaluations before. However, improving data availability might influence reference species lists and/or reference levels due to new insights. Occasional validation of reference values might be necessary during the years. As indicated in the OSPAR CEMP (OSPAR, 2019), the identification of indicator species based on 'typical species composition' in uni-pressure to reference area comparisons is highly valuable and is proposed to function as input for the selection of indicator species and reference values when available for the (sub)region and ecotope of concern.

Table 6.3.3.a. Overview of the various BISI evaluations and recommendation for the species specific indicator values (iv). Besides the general quality evaluation, these include specific evaluations to identify potential causes for the observed quality status and potential effects of the observed quality status on ecosystem functions. Categories of specific evaluations also equal the criteria that were considered for indicator species selection at which potential indicator species should score well (Scoring an iv_i of 1 for A or B or another more important pressure in the area of concern, or an iv_i of 1 for F, or a score of at least 1.5 for the combined categories C+D+E).

Code	Causes and effects (to be evaluated)	Description	Species specific indicator value (iv_i)
General quality		Selected indicator species according to the five criteria described in the text: Potential indicator species for relevant ecotope; either characteristic, indicative for a dominant disturbance or potential sufficient large, old and/or having frequent recruits and present in vicinity; specific enough towards quality status so that the power of testing is sufficient good.	1 (by definition)
A.	Sea floor disturbance	Combined indicator value for a variety of disturbances (different types, intensity and/or frequency).	5 levels (0, 0.25, 0.5, 0.75, 1)
B.	Ecological disturbance	Combined indicator value for effects of nutrients, pollutants and toxicants, and potentially hypoxia and temperature increases.	5 levels (0, 0.25, 0.5, 0.75, 1)
C.	Intensity of sea floor disturbing fisheries	Indicator value on basis of size of species (where large species can be damaged or fished away at low intensity of sea floor disturbing fisheries and smaller size classes only at high intensity of sea floor disturbing fisheries).	4 levels (0.25, 0.5, 0.75, 1)
D.	Frequency of sea floor disturbing fisheries	Indicator value on basis of age of species (species that get older are already impacted at a low frequency of sea floor disturbing fisheries, whereas species that live shorter are likely only impacted by frequent occurring sea floor disturbing fisheries).	10 levels (age divided by 10, value of 1 at age >10)
E.	Recovery	Indicator value on basis of frequent recruits (Species with frequent recruits are good indicators for the first phases of recovery).	4 levels (0, 0.1, 0.5, 1)
F.	Characteristic species	Species are almost exclusive or are much more abundant in the area of evaluation than elsewhere (identification of being characteristic at ecotope level is a criterion for indicator species selection).	3 levels (0, 0.5, 1)
G.	Food web structure	Species important as food sources for higher trophic levels (i.e. fish, birds, marine mammals).	3 levels (0, 0.5, 1)
H.	Habitat diversity	Species creating permanent structures providing niches for a range of additional species.	4 levels (0, 0.25, 0.5, 1)
I.	Biological activation of sea floor top layer	Bioturbating and bioirrigating species with an important role towards ecological functioning (e.g. nutrient cycling, degradation of pollutants, providing suitable habitat for other species).	5 levels (0, 0.25, 0.5, 0.75, 1)
Specific evaluations already in use in the Netherlands for evaluation of certain areas or habitats: - Habitat Directive typical species - Indicators of increased hydrodynamics			

- Indicators of increased mud content
- Indicators of increased inundation duration
- Indicators of seafloor subsidence
- Indicators of indirect effects of sediment extraction and – suppletion (increased turbidity water column)

Other evaluation that might be of interest:
- Indicators of impacts from exotic species
- Indicators of temperature increase

In case of the BISI for the Dutch North Sea based on v1, expert judgement of several experts was considered to select 'smart species' (Wijnhoven et al., 2013). At least all 'typical species' as identified in the Netherlands for Habitat Directive Annex I habitats H1170 (reefs) and H1110 (Permanently submersed sandbanks) (Min EZ, 2014a,b) were considered, but ultimate selection was based on comparable criteria as indicated above and described in Wijnhoven & Bos (2017) and the associated BISI protocol v1.

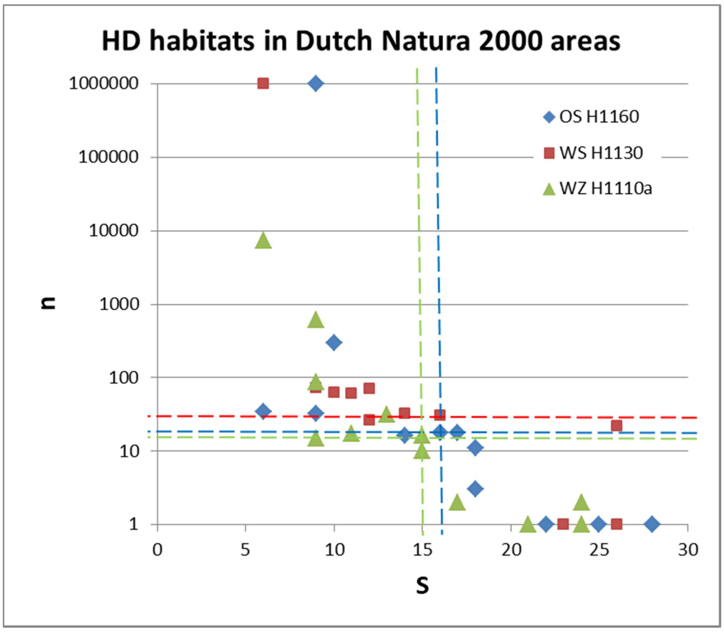
A BISI consists of an overall quality evaluation (general quality index) for which indicator species selection is standardized as described before. A general quality evaluation comes with a series of specific quality evaluations (specific indices) to detect possible causes and/or effects of observed quality levels and/or quality developments (Table 6.3.3.a). Basically the specific evaluations consist of the same indicator species selections as the general BISI, selecting those species relevant (with an indicator value larger than zero ($iv_i > 0$) for the specific evaluation (see example indicator species selection and valuation in Table 6.3.3.b). It is allowed to add additional species to the specific evaluations (evaluations are therefore less standardised and less comparable between different areas than the general BISI-scores), to enlarge the number of indicator species in the specific evaluation. These are likely indicator species not considered general quality indicators that might however be indicative for certain very specific functions and/or pressures and certain species selections might be of specific interest as they have a certain status in management. This includes for instance the 'HD typical species' (in the Netherlands) or other species with official status (in other countries) that do not reach the criteria to be selected for the general quality status. Power analyses should give insight in the power of the tests regarding specific target evaluations and in which timeframe significant results might be expected if present, after which an option might be to change sampling efforts, frequency and/or design.

Ideally, the power of specific evaluations is calculated and presented with the results. The identified specific evaluations for BISI v1, partly in use as criteria for indicator species selection for the general quality evaluations as well, are presented with their suggested levels of scoring in Table 6.3.3.a. It is likely that with the development of BISIs for new areas, a need for additional specific evaluations (e.g. additional pressures of importance) arises. Those can easily be constructed and defined in a similar way based on the same (sub)set of data. Other specific evaluations presented here, are possibly less relevant, and can be omitted, except for specific evaluations C, D, E and F that have a role in indicator species selection for the general quality evaluation, and A and/or B that should than be replaced by the most important pressure(s) for the area of concern.

The evaluation of indicator species characteristic for a certain ecotope compared to species characteristic for alternative ecotopes (especially in areas where changes are expected or desirable) can be considered a specific evaluation as well. Basically such results will become visible in case areas are in change (often as a result of human related activities like large scale constructions; e.g. windfarms, artificial islands, sand extractions and suppletions, dikes, embankments and construction extensions into the sea) or large-scale developments (sea level rise or changed currents due to climate change or seabed lowering due to gas extraction), as BISI methodology is based on a static ecotope map. When large changes in the seabed habitat constitution are expected; changes in BISI scores can be compared to ecotope-difference maps (comparing ecotope surface area changes in time).

With the construction of BISI v1, it was suggested that indicative a specific BISI should consist of at least 5 indicator species for a reliable outcome. The realized power will however be the result of the number of indicator species, the number of samples, and the sensitivity and specificity of species related to certain specific evaluations. So increasing the number of indicator species might to certain extend lead to improved power, as long as added species are really sensitive and specific; otherwise they will only lower the power. Also samples should give a representative view of the area of concern; the number of samples needed will be lower in case of a clearly distinguished ecotope (instead of a composite area), and when taken in a short period of time (including different seasons and/or different years will lead to larger (natural) variability in the data). Additionally, increasing the spatial scale will increase the variability due to the introduction of naturally occurring geographical differences in indicator species occurrences. So introducing additional (spatial or temporal) variability in the data will lead to the need of more samples to achieve a certain power. Additionally the power depends on the kind of test and the level of quality status to compare with (it is easier to detect differences from a clearly distinguished quality status, i.e. very high or very low, than when several indicator species are present in average occurrences). This makes that we can only give an indication of the number of indicator species and samples that should be strived for and that it is necessary to test on a regular basis for the achieved power at the ongoing quality status.

Testing (bootstrap methodology selecting sets of samples – with return of samples – from relative large actual datasets) in a variety of systems with regards to the development of BISIs for evaluation of HD habitat (sub)types within Natura 2000 areas in the Netherlands (i.e. composite areas consisting of different ecotopes, however with a certain commonality as they are part of the same HD habitat (sub)types) indicates that with about 15 to 16 indicator species in these areas the optimal power is reached.



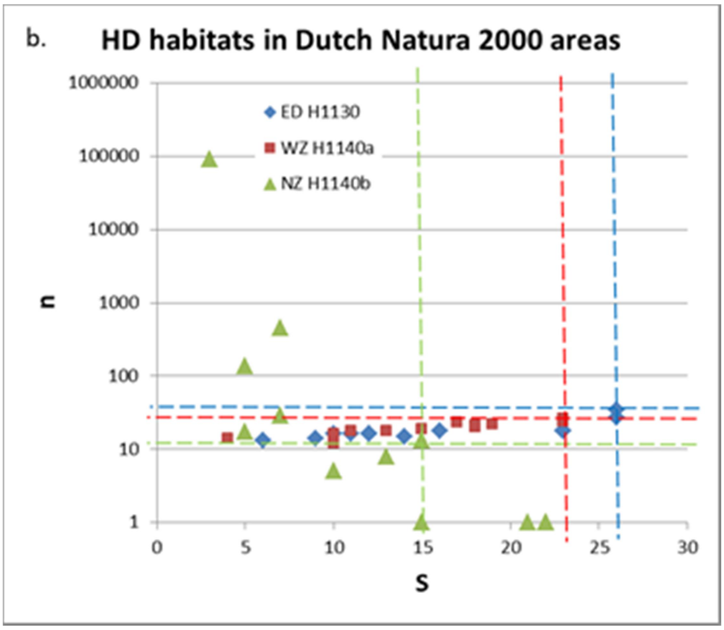


Figure 6.3.3. Example showing the relation between the number of indicator species in the BISI (S) and the calculated average number of samples (n) needed to achieve a power of 0.8 in detecting 50% differences in the BISI-score at $p<0.05$. Shown are the results of general and specific evaluations (differing in the number of indicator species included) for Habitat Directive habitats in Natura 2000 areas: a) H1160 in the Eastern Scheldt (OS), H1130 in the Western Scheldt (WS) and H1110a in the Wadden Sea (WZ); b) H1130 in the Ems-Dollard (ED), H1140a in the Wadden Sea (WZ) and H1140b in the coastal zone of the North Sea (NZ). Calculated from Wijnhoven & Van Avesaath (2019).

A number in the range of 13 to 30 samples is needed than to allow detection of 50% differences in BISI-score with a power of 0.8 ($p<0.05$). The results in Figure 6.3.3 also show that a reduction in the number of indicator species does not lead to a significant decrease of the power for several (specific) evaluations; dependent of the (natural) variability within the system. BISIs based on 8 (e.g. H1140b in the North Sea) to 13 indicator species (H1130 in the Western Scheldt) do still result in about a similar power for all tested (specific) evaluations. It has to be noticed that it is very well possible, that certain (specific) evaluations are less relevant and/or selected indicator species do appear to be less sensitive/indicative in the system of concern, than expected. This might be the case for some of the specific evaluations where it is found that larger numbers of samples are needed, or in case of H1130 in the Ems-Dollard and H1140a in the Wadden Sea, where tests with lower numbers of indicator species do not lead to reduced power (it seems that the indicated 27 and 23 indicator species needed for sufficient power for each of the specific evaluations includes less indicative species, and that selection of 5 to 10 of the most indicative species leads to at least a similar and possibly a larger power.

With the design of the monitoring programme (for each new area) one should on beforehand determine the targets of evaluation. For a general quality evaluation a limited number of samples can already be sufficient, whereas when identification of the potential role of certain pressures is essential, a larger number of samples might be requested. However, also the time-frame in which results are needed, can influence the necessary number of samples, and can be a way to reduce sample numbers (per year). One should however always determine the realized power of constructed BISI's for new areas to be evaluated, to identify the necessary number of samples in line with the prevailing situation (quality status), as the power will deviate with indicator species relative occurrences.

Table 6.3.3.b. Example of indicator species selection at the level of ecotopes with indication of used sampling technique (evaluation based on monitoring with different techniques). First five indicator species* of BISI for HD habitat type H1160 shown with indication of ecotope specificity (value between 0-1; in case >0.5 species indicative for specific ecotope) and indicator value (iv_i) towards different specific evaluations (value between 0-1). All indicator species are included in the evaluation of the general quality status of the benthic habitats (iv_i=1), whereas species occurrence can be indicative for the different pressures effects, for recovery, or for possible effects of the observed quality status on aspects of ecological functioning. Total number of indicator species included in the BISI (specific) evaluations with regards to the quality status of H1160 in the Netherlands is indicated. An entire area (such as in this case the HD area Eastern Scheldt qualified towards H1160) can be evaluated by combining ecotope specific references surface ratio based (or evaluations can take place at the level of individual ecotopes).

HD habitat type H1160	Ecotopes (ZES.1)						Pressure indicator								Recovery	Ecological functioning			
	Sublitoral High-dynamic	Sublitoral Low-dynamic Deep	Sublitoral Low-dynamic Shallow	Litoral High-dynamic	Litoral Low-dynamic	Sampling technique	Indicator species general quality	Characteristic species	Sand hunger: Increased hydrodynamics	Sand hunger: Increasing inundation	Ecological disturbance	Seafloor disturbance	Size potential (fisheries intensity)	Longevity (fisheries frequency)	Frequent recruits potential	HD typical species	Foodweb importance	Habitat diversity importance	Biological activation importance
Indicator species	Z2.11	Z2.122	Z2.123	Z2.21	Z2.22		Gen	F	K	M	B	A	C	D	E	J	G	H	I
Arenicola sp.	0	0	0,25	0,25	1	(Box)core	1	1	1	1	0	0,5	0,75	0,6	1	1	0	0	1
Bathyporeia pilosa	0,25	0	0	0,25	1	(Box)core	1	0	0,5	1	1	0,5	0,25	0,1	1	0	1	0	0
Bathyporeia sarsi	0,25	0	0	0,25	1	(Box)core	1	0	0,5	1	1	0,5	0,25	0,1	1	0	1	0	0
Carcinus maenas	0,5	0,5	1	0,5	1	(Box)core	1	0,5	0,5	0	0,5	0	0,75	0,6	1	1	0,5	0	0,5
Cerastoderma edule	0	0	0,25	0,25	1	Dredge/grab	1	1	1	1	0,5	0,5	0,75	0,9	0	1	1	1	0,5
Indicator species (n):	11	11	17	2	17		25	9	16	8	13	16	25	25	21	9	17	5	14

*Also other taxonomic levels can be used when species show about similar sensitivity (in case data from different sources or campaigns are used, it is of importance that methodologies (including taxonomic identification) are aligned as much as possible; sometimes this can only be achieved using a higher taxonomic level for certain groups.

6.3.4 Evaluation relative to compiled reference levels

There are pros and cons for using reference levels in evaluations. A pristine (pre-industrial) reference level reflecting the situation before anthropogenic disturbances will reflect an optimum quality situation. Even if we reach (or reconstruct) pre-industrial environmental conditions, it is unlikely that the original community will return, even in the long term. Taking away the pressures will likely not lead to recovery of pristine benthic communities, as habitat characteristics and present species pools have changed dramatically, which makes that natural development under high environmental quality conditions will lead to alternative stable states. Therefore, a methodology is needed which is potentially capable of showing improvements on the short and mid-long term, so that effects of management regulations can be detected within management cycles. Unrealistic reference levels would mask changes (if there are) as relative differences between reference levels and observations would be minimal.

A benefit of working with reference levels is however, that changes are put in perspective. As an example: Doubling of the observed numbers for one indicator species might be much more important than for the other, as such an increase can mean that the abundance of the indicator species is still at a poor quality level or comes at a level that no further quality improvements are expected/necessary.

For each area to be evaluated, an area specific (internal) reference is constructed. With BISI v2, internal references are specific for ecotopes and can potentially be used for different areas where the same ecotope is present. Exceptions are potential indicator species that in certain regions have no opportunities of colonizing the area of evaluation in a natural way; those indicator species should be excluded or replaced by region specific indicator species. For evaluations of other types of areas and habitats, internal references are compiled from the references of the (most important) ecotopes, surface ratio based.

The flow chart of Table 6.3.4.a shows the proposed standardized decision scheme for derivation of ecotope related reference values. Reference derivation methodology is dependent of the presence of suitable reference areas, historic data availability, the area monitored, the monitoring methodology used, the within data variability, and how recent observations (year of evaluation) compare to recent historic observations.

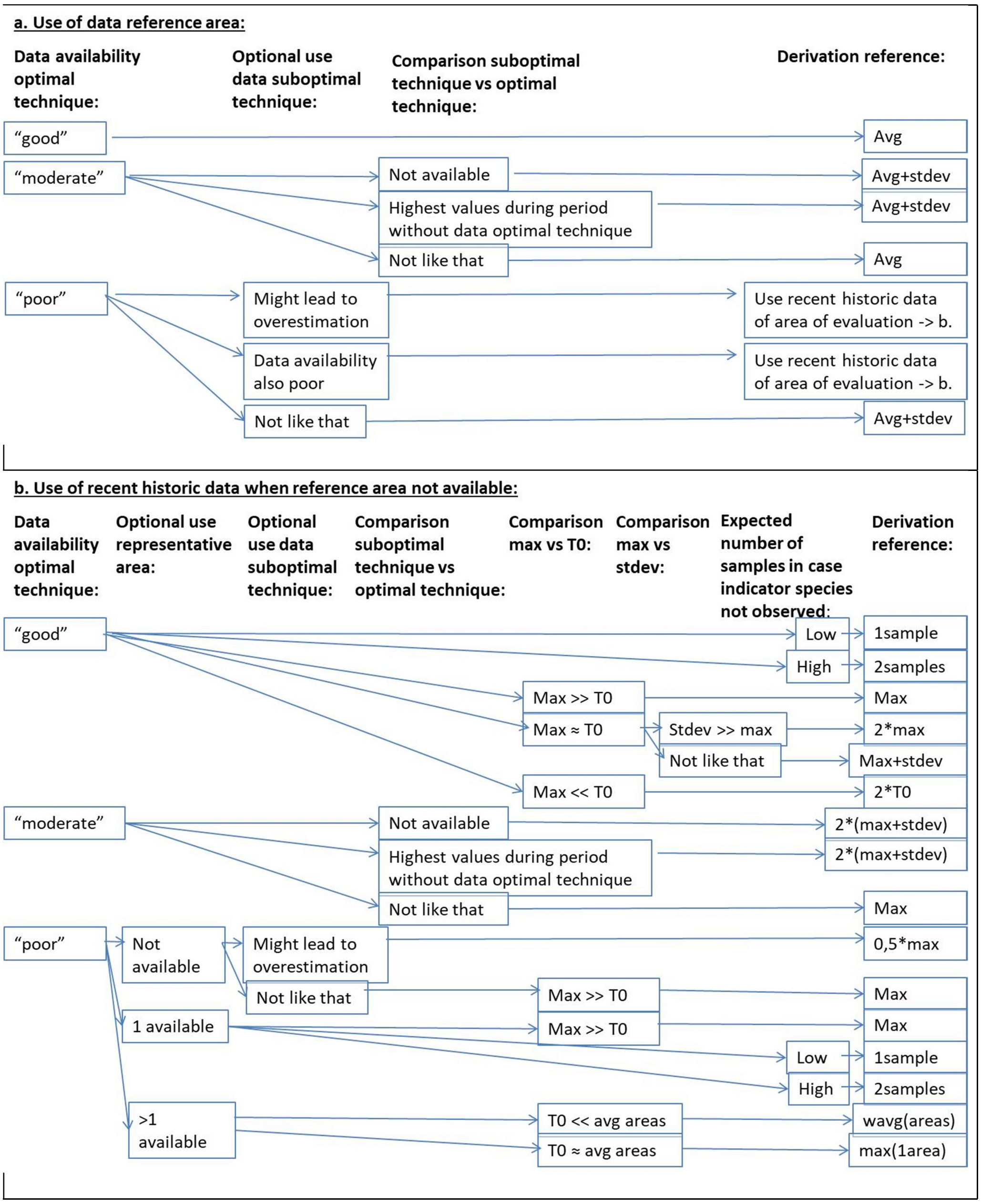


Figure 6.3.4.a. Flow chart showing the decision schemes of how to derive indicator species and area specific reference values. a. Scheme to use in case a reference area is available; reference values can potentially be obtained from monitoring data of a reference area (e.g. results from typical species composition identification in uni-pressure - to reference area comparisons according to OSPAR BH1 (OSPAR, 2019). b. Scheme to use in case no suitable reference area is available, or if monitoring data for the reference area are too poor. Avg = representative year average value in this case for a reference area; Max = maximum year average value in this case in recent historic data; stdev = standard deviation; T0 = year average value as observed in the focal year for which an evaluation methodology is developed and to which future evaluations will be compared; 1sample = a density similar to an occurrence in one sample; wavg = weighted average of several areas based on the number of samples taken per area; >> = much larger; << = much smaller; ≈ = comparable values.

The use of data from reference areas is an addition to the methodology in BISI v2. As the derivation of reference values and the construction of an internal reference for areas to be evaluated are ecotope based, in practice data from corresponding ecotopes are searched for in reference areas, after which a suitable reference might be constructed surface-area based. Such references should be from a comparable region, i.e. preferably part of the same regional species pool but at least from the same geographical zone. As explained before, potential indicator species that cannot return to the area of evaluation in a natural way are excluded from the internal reference.

Therefore reference levels are either obtained from recent data of reference areas for which the possible presence of significant pressures can be ruled out, or are based on recent maximum observations of year averages that are adapted, doubled and/or increased with the standard deviation as observed from recent historic monitoring data of the area of evaluation itself. Derivation methodology depends on the historic data availability. In case of poor historic data availability, there are no better options than using maximum observations from suboptimal techniques, in which case a reference of half the observed maximum is sometimes used. There are cases that indicator species have not been present in the monitoring data of the area of evaluation (as densities were too low) during recent years. In such cases a presence of 1 specimen per square meter in only 1 or 2 of the samples is used as a reference (dependent of the expected sampling efforts during monitoring the coming years).

Table 6.3.3.c. Example of internal reference occurrences as in this case used for the BISI of HD habitat type H1160, defined at the ecotope level (codes refer to ecotopes according to ZERS.1 as indicated in Table 6.3.3.b) and calculated into reference occurrences for the Eastern Scheldt, surface ratio based. Only the first five indicator species of the BISI for H1160 are shown. Reference values are monitoring technique and sampled surface specific, although different techniques can be used for different species or even combined for one species in case sampling strategy is consequent in the evaluation/comparison (e.g. specific technique in specific habitat or established ratio).

HD habitat type H1160		Reference (R_i in n/m^2) R_i = (0,262*Z2.11) + (0,160*Z2.122) + (0,286*Z2.123) + (0,023*Z2.21) + (0,270*Z2.22)					
Indicator species	Sampling technique*	Eastern Scheldt	Z2.11	Z2.122	Z2.123	Z2.21	Z2.22
Arenicola sp.	(Box)core	53,093	3,175	20,290	31,206	80,000	142,029
Bathyporeia pilosa	(Box)core	86,865	5,128	0,000	1,515	31,847	313,044
Bathyporeia sarsi	(Box)core	41,142	25,926	0,000	5,790	233,333	101,449
Carcinus maenas	(Box)core	21,837	9,091	8,333	37,879	20,000	25,397
Cerastoderma edule	Dredge/grab	106,807	10,000	20,000	61,121	163,924	296,034

**Use of standard boxcore (0,078 m^2) or handcore (0,0157 m^2) and dredge (0,1 m^2) or grab (1,06 m^2) dependent of habitat type.*

Although there is expert judgement involved (and it is allowed to deviate from the proposed derivation scheme in case of good reasons), the derivation of the reference values is standardized as much as possible.

Table 6.3.3.c shows an example of an internal reference used in the BISI (only 5 species shown), defined at the level of the identified ecotopes and calculated surface ratio based into reference occurrences at the level of an area or habitat to be evaluated. Species occurrences can be spatial occurrence (presence/absence), densities or biomass. References are however sampling technique and sampled surface specific. Identified internal references at the ecotope level are basically generic, and can potentially be used for other areas of the same type (in the example of Table 6.3.3.c 'large shallow inlets and bays'; H1160) in the same geographical (sub)region. Monitoring programmes should match the purpose of the evaluations (give a representative view of the quality status of areas or aspects of concern); and selection of the suitable monitoring data might be necessary (see chapter 7 Determination of GES and boundaries). In case monitoring programmes are not representative for entire (samples reflecting surface ratios) regions, evaluation of solely certain habitat types (preferably important ones that might be indicative for the status in the entire area) might be an option.

Besides indication of the methodology used for species observation, it is of importance to identify which specimens[4] belong to the numbers per species. This is often a decision already taken in the sample identification phase (which makes that it is of importance that standard protocols for identification are used). Basically only the specimens identified to the species level as indicated in the reference list are used for evaluation (where it is indicated when especially in the historic data or in evaluation protocols other names for the same species have been used, as well). This can mean that for instance juveniles (often only identified to the genus level) are generally not considered which is perfectly fine as those are much more susceptible to seasonal or occasional fluctuations. It has to be taken into account that the sampling methodology used, and the mesh-size in particularly, determines which specimens to consider. Tiny specimens are likely under sampled and should therefore not be part of the numbers to be considered, so that selection of specimens from the observation data on basis of size (species specific as the effectivity of the methodology is besides size also shape related) might be necessary.

[4]Are only those specimens identified at species level included, or are optionally other taxonomic levels included in case no other related species are present (e.g. to include juveniles)?

6.3.5 Calculation of BISI-values

Next step in the evaluation is defining the occurrence (O_i) of indicator species for the year(s) and area(s) of evaluation based on the occurrence data (methodology, data type, sample type) as indicated. The occurrence is evaluated taking the observed variance into account. The occurrence to reference ratio (O_i/R_i) is calculated for each of the indicator species. To downscale the effect of possible extremes in the observation data, O_i/R_i ratios are truncated at 0.01 and 100 (i.e. 100x an improvement or degradation of the observation compared to the reference). The observed standard deviation (as a measure of variance) is calculated into a value relative to the calculated Oi/Ri ratio. Similarly as for the O_i/R_i ratios, the adjusted standard deviation is set to a value of 0.01 in case the observed standard deviation equals zero (Table 6.3.5.a).

Table 6.3.5.a. Example of calculation (with or without truncation as visible in the adjusted value) of the occurrence to reference ratio (O_i/R_i) with accompanying standard deviation. Here data from the Eastern Scheldt in 2012 are used as the observation data (only first five indicator species shown), where calculated results from the different monitoring techniques are indicated in different colors.

HD habitat type H1160		**Eastern Scheldt**						
Indicator species	Sampling technique	**R_i**	**O_i** (2012)	±stdev	**O_i/R_i** (2012)	adjusted O_i/R_i	±stdev	adjusted stdev
Arenicola sp.	(Box)core	53,093	8,381	36,787	0,158	0,158	0,693	0,693
Bathyporeia pilosa	(Box)core	86,865	0,000	0,000	0,000	0,010	0,000	0,010
Bathyporeia sarsi	(Box)core	41,142	3,352	14,414	0,081	0,081	0,350	0,350
Carcinus maenas	(Box)core	21,837	1,676	10,333	0,077	0,077	0,473	0,473
Cerastoderma edule	Dredge/grab	106,807	7,333	16,676	0,069	0,069	0,156	0,156

Besides a general quality assessment (in which all area, ecotope or habitat specific indicator species equally participate, and meet the selection criteria of having sufficient power, and are therefore considered 'Smart species'), specific evaluations are performed on weighted species subsets. In BISI v2 indicator species lists are optionally supplemented with additional species (depending on the specific indicator value of species for certain causes of change and potential effects of change).

Therefore the adjusted O_i/R_i ratios are multiplied with the species weights (IV_i's calculated as iv_i divided by iv_{avg} as indicated in the example of Table 6.3.5.b). To calculate the geometric mean of sets of indicator species results, the natural logarithm is taken from the product. This will downscale the importance of extremes, put emphasis on the presence or absence of indicator species and make relative improvements and degradations of equal importance. Individual indicator species results (IIS_i) are multiplied with the number of samples (which can deviate among species due to the optional use of different monitoring techniques for different species).

Table 6.3.5.b. Example of calculation of the BISI including standard deviation. Adjusted O_i/R_i ratios at the level of individual indicator species (as calculated before: E.g. Table 6.3.5.a) are multiplied with the species and evaluation specific indicator value ($IV_i = iv_i/iv_{avg}$), where the natural logarithm is taken from the result. The results multiplied with the number of samples (can differ per indicator species due to use different monitoring techniques) are summed and divided by the average number of samples per indicator species in the evaluation, after which the inverse natural logarithm (e to the power of the product) of the result divided by the number of indicator species (in the evaluation) is taken (back transformation) which delivers a BISI value (between 0.01 and 100). The accompanying standard deviation (only results are shown) are calculated by multiplying the squared number of samples with the squared adjusted standard deviation (= variance) per individual indicator species divided by the squared O_i/R_i ratio. The sum of the results per individual indicator species is multiplied with the squared BISI to number of species ratio $(BISI/S)^2$. The square root of the product (which is the variance) is the standard deviation belonging to the calculated BISI. Here data from the Eastern Scheldt in 2012 are used as the observation data (only first five indicator species of the in this case (i.e. Table 6.3.3.b) up to 25 indicator species are shown).

HD habitat type H1160	$n_i*IIS_i = n_i*\ln((iv_i/iv_{avg})*(O_i/R_i))$												
	Pressure indicator								Recovery	Ecological functioning			
			Sand hunger										
	Indicator species general quality	Characteristic species	Increased hydrodynamics	Increasing inundation time	Ecological disturbance	Seafloor disturbance	Size potential (fisheries intensity)	Longevity (fisheries frequency)	Frequent recruits potential	HD typical species	Foodweb importance	Habitat diversity importance	Biological activation importance
Indicator species	Gen	F	K	M	B	A	C	D	E	J	G	H	I
Arenicola sp.	-70,152	-63,224	-56,916	-67,980	na	-76,009	-54,059	-62,951	-69,278	-70,152	na	na	-55,040
Bathyporeia pilosa	-174,996	na	-188,100	-172,824	-162,211	-180,854	-200,651	-235,883	-174,123	na	-170,521	na	na
Bathyporeia sarsi	-95,280	na	-108,384	-93,108	-82,494	-101,138	-120,935	-156,166	-94,407	na	-90,804	na	na
Carcinus maenas	-97,550	-116,961	-110,654	na	-111,103	na	-81,457	-90,349	-96,676	-97,550	-119,414	na	-108,777
Cerastoderma edule	-40,179	-37,444	-34,954	-39,322	-45,529	-42,491	-33,827	-31,255	na	-40,179	-38,412	-38,874	-44,611
$BISI = \exp((1/S)*\sum\ln(IV_i*(O_i/R_i)))$	**0,074**	**0,049**	**0,049**	**0,077**	**0,066**	**0,067**	**0,061**	**0,046**	**0,090**	**0,087**	**0,066**	**0,056**	**0,117**
± stdev	1,737	2,029	1,598	3,547	2,025	1,802	1,401	1,056	2,337	3,503	2,050	2,432	4,023

In a similar way the accompanying variance (terms are squared as the variance is equal to the squared standard deviation) is calculated by multiplying with the number of samples and dividing by the occurrence to reference ratio: n_i*$Stdev_i^2/(O_i/R_i)^2$. The used indicator value per individual indicator species (IV_i), is lost in the deviation as it will appear in both the numerator and denominator.

From these values, the general and specific Benthic Indicator Species Indices (BISIs) can be calculated according to:

$$BISI = \exp((1/S)*((\Sigma n_i*\ln(IV_i*(O_i/R_i)))/n_{avg}))$$

To simplify the equation the aspect of taking into account the number of samples is not written in the resulting BISI equation:

$$BISI = \exp((1/S)*\Sigma \ln(IV_i*(O_i/R_i)))$$, where

S = Number of indicator species included

IV_i = Indicator Value calculated as iv_i (species specific indicator value with a value between 0-1) divided by iv_{avg} (the average indicator value of all indicator species (with $iv_i>0$) in the specific evaluation.

O_i = Observed occurrence (ratio of samples with the indicator species present) or observed numbers (average densities)

R_i = Reference occurrence (set ratio of samples with indicator species present under reference condition) or observed numbers (set average densities under reference condition).

(exp = the inverse natural logarithm (e to the power of the formula) as a back-transformation of the transformation according to the natural logarithm (ln)).

The general BISI is a value for the general quality status of the area, ecotope or habitat to be evaluated. Basically the BISI can be tested on significance against the compiled reference which provides a quality state estimation that can be used. Working with the BISI making use of an as much as possible standardized internal reference, potentially provides options to set standardized threshold values for the quality status to strive for. A BISI-value of at least 1 could be the ultimate target. Taking uncertainty (e.g. as accepted due to working with a preferred number of samples sufficient to allow to detect at least 50% changes in BISI-values, however also accepting that smaller quality changes might be undetected) into account, a suitable threshold for management applications and quality evaluations (e.g. with regards to the MSFD) could be a BISI-value of at least 0.5 for a sufficient good quality status on the medium long-term. It should be identified what can be a realistic time-frame to achieve sufficient quality improvements when dominant pressures have been decreased significantly to minor levels with minimum to no impact. This is habitat and community dependent but in case of recovery after disturbance only (leaving substrate characteristics largely intact) this should be in terms of years to tens of years (considering indicator species life-cycles including amongst others reproduction, longevity and settlement opportunities dependent of biological activity already or still present); but is for instance shorter in soft sediment habitats than for reefs. Thinking in MSFD cycles; 6 years of recovery (after successful measures) can be sufficient in relatively fast recovering habitats; 12 years is more realistic for most habitats; and in certain cases 18 years (or even more) might be necessary.

The methodology is specifically geared to evaluate potential changes in time. Related to management the future quality status is preferably compared to an initial situation; a T0 of the situation before measures are taken, or the situation at which it was decided that the area or habitat of concern should be conserved or is in need of quality improvement (in line with amongst others MSFD and HD evaluation cycles).

Initially the BISI methodology was developed to compare the quality status of sea areas to a certain initial situation (T0). Specific evaluations are predominantly intended to identify the relative importance of potential causes or the impact of changes in functions (effects), on basis of relative differences in specified BISI values and the significance of potential differences relative to the T0 and developments in time in (specific) BISI values.

With BISI v2 where the design of the area specific index is more standardized, the comparability of quality evaluations between different areas is improved. Compilation of the area specific index has become ecotope based, using the same references for the same ecotope in different areas. Application in various circumstances and different areas, including possible reference areas, can support fine-tuning of the current methodology. It should be taken into account that results will always be impacted to some extent by aspects that are out of reach of a methodology development: E.g. data availability, representability of monitoring (techniques and efforts), habitat constitution (concealed by aggregated large-scale habitats), and etcetera.

6.3.6 Evaluation of quality status and significance testing

Basically with having the general - and specific BISI values for areas, ecotopes and habitats for moments of evaluation, the results of a quality assessment are there. It however depends on the type of reporting/evaluation (as for instance indicated in Figure 6.3.1.a), which quality data to test, combine or consider and what a good quality status looks like.

With BISI v1 the assessment methodology was specifically developed at the level of management areas like in case of the Dutch North Sea, evaluation of Areas of specific ecological value (ASEVs including Natura 2000 areas), Habitat Directive habitat types (that include (parts of) ASEVs but generally extent outside ASEVs), and EUNIS ecotopes (that cover areas partly inside and outside ASEVs). With BISI v2 indicated assessment areas can still be core targets for evaluation. However, ecotopes (former EUNIS level 4 (EMODnet, 2016) or MSFD broad habitat types (proposed EUNIS level 2; EUSeaMap, 2019) or comparable ecotopes like aggregations or according to other ecotope classification like ZES.1 (Bouma et al., 2005)) form the basis to construct area specific evaluation indices using the surface area ratio of ecotopes in the area of evaluation. Herewith the assessment indices, once available at the level of ecotopes, can be converted into specific indices for any area, once classifiable into (several of) those ecotopes.

Besides the evaluation of the quality status of specific areas, the evaluation methodology for the Dutch North Sea region came with special evaluations according to a BACI-approach to test the efficiency of management measures on basis of a comparison of (partly) for specific fisheries closed and open areas. This is actually a matter of design and the way of testing, but the used BISIs are the same: I.e. based on ecotope composition of the area of concern or composed on basis of monitoring location distribution ratio over the distinguished ecotopes.

For each of the indicated areas to be evaluated the objective can be to achieve conservation of the current quality status (i.e. no decrease in the quality status) or an improvement of the quality status (for which there might be a timeline). As indicated, BISI levels can become management targets as well; e.g. like the installation of threshold values, preferably after testing and broadscale application in similar areas and if available including reference areas.

Due to changes in methodology with BISI v1 a BISI-score of around 0.736 (similar to the internal reference) is considered a good quality status, whereas with BISI v2 the scale is adjusted so that a BISI-score of around 1 is considered a good quality status. As indicated, management targets can also be different values. Considering accepted uncertainty and the detectability of quality differences (power of tests) a BISI-value of 0.5 with v2 could be a suitable threshold value for a sufficient good quality status to be achieved on the mid-long term. Also evolution in the direction of a BISI-value of 1 or 0.5 (in case of observed poor quality status) for those areas and habitats with a conservation objective might be beneficiary from a nature perspective (ecological functioning) and to safeguard natural resources. In other European regions with low pressure levels a BISI-score of below 1 is possibly not a good objective as it might involve a decrease in the quality status; such a decrease should only be allowed for very good reasons.

HD habitat type H1160	Indicator species general quality	Sand hunger	
		Increased hydrodynamics	Increasing inundation time
	Gen	K	M
BISI	**0,074**	**0,049**	**0,077**
±stdev	0,049	1,598	3,547
2-sided independent t-test (with reference)			
Pooled Std Dev	0	2	4
Computed t Statistic	394,248	10,108	3,166
Critical Value of t	1,961	1,962	1,964
Probability of Computed t	0,000	0,000	0,002
Significance	***	***	**
2-sided independent t-test (among specific evaluations)			
Pooled Std Dev		2,258	2,584
Computed t Statistic		-0,129	0,050
Critical Value of t		2	2
Probability of Computed t		0,897	0,960
Significance		ns	ns

Table 6.3.6.a. Example of significance testing for the example of the Eastern Scheldt in 2012. Comparing BISI results of 2012 with the internal reference; quality status as observed significantly lower than the internal reference for all tests (general quality evaluation and both specific evaluations). Comparing the importance of the impact of increased hydrodynamics and increased inundation time on the current quality status; although the observed impact of the first on the general quality status might be slightly larger; this is found to be not significant (both aspects of sand hunger are about of equal importance and play a significant role.

In case of comparison with the internal reference, the Pooled standard deviation is equal to the calculated standard deviation accompanying the BISI for the observation case. The t statistic is calculated by taking the difference of the BISI values (in case of comparison with the reference this is 1-BISI) divided by the square root of the sum of squared standard deviations to sample number ratio. In case of comparison with the reference this could be (1-BISI) divided by the square root of 2 times the standard deviation ro sample number ratio. The critical t-value equals the nnverse of the two-tailed Student's t distribution calculated in Excel with the T.INV function considdering the degrees of freedom (in this case the sum of 'species x number of samples' – 2, which in case of a comparison with the reference is 2 times the same number. The probability of the computed t is than calculated by considdering a t-distribution (T.VERD in Excel) over the absolute value of the computed t statistic, considdering the degrees of freedom.

Basically, all kind of testing can be applied to BISI results, as it provides average values with standard deviations optionally for various situations, areas or moments in time. Obvious tests are 2-sided independent t-testing of years of evaluation against the reference (or 1-sided if it is obvious that the quality status is below the reference level) to evaluate whether possible observed differences can be considered real differences. Additional 2-sided independent t-testing of the quality status of different areas or the same area in time; that can be a 2-sided paired t-test (e.g. compared with aT0 situation before management regulation are taken) in case of a fixed monitoring design and recurrent sampling at the same sites. After several observation moments in time (several years of monitoring; indicative at least 5) trend analyses can indicate possible changes in quality status and quality status development optionally including testing whether developments are (partially) independent of natural or large-scale developments outside the influence of management measures. The independent t-tests are already integrated in the provided Assessment Tools, so that results immediately come available when observation data (possibly with changed monitoring efforts) are entered.

A special case is the testing of differences in developments according to a BACI design as for instance is the case in the comparison of for specific fisheries closed and open areas in the Netherlands with a dedicated monitoring programme. In such an evaluation potential initial differences in benthic indicator species assemblages (at T0) are taken into account. To avoid an effect of initial differences, the difference in singular indicator species BISI values is calculated between the two areas with different treatments on T0 and the other moment of evaluation. The average difference in BISI with accompanying standard deviation is calculated from the results of individual species. Results in BISI-differences between different treated areas are compared between T0 and the other moment of evaluation using a one-sided t-test.

6.4 Assessment benchmark

Dependent of the area, ecotope or habitat to be evaluated, a consolidation of the current (T0) BISI values or an increase in BISI values indicates a good (or desirable) quality status for the Dutch situation. Such an increase has to be significant (at $p<0.05$) taking natural fluctuations into account. Good Quality Status is when (certain) pressures are effectively reduced and lead to increasing index values. I.e. when management measures initially result in increasing BISI values in 'for specific activities closed' areas and might lead to an increase in BISI values for an entire area on the longer term.

However, with regards to the MSFD and broader standardization at the national level, there is a request to work with threshold levels. With the current methodology and the as much as possible standardization of obtaining internal references; BISI results within the same ecotopes, but also BISI results among different ecotopes or composite areas, will be comparable to a large extent. This is the case when monitoring efforts are comparable; monitoring is representative for the area of evaluation and when data availability for derivation of reference levels is comparable; i.e. sufficient for each of the ecotopes of concern. All aspects might leave room for improvements.

The derivation of internal reference levels, although monitoring efforts, representivity and disturbance levels are considered, will definitely profit from increased data availability, testing and application. With regards to generic application at the regional/European scale, it is of importance that insights from other geographic parts of the regions of concern are achieved. The accuracy of the indicator might significantly improve when data from reference and low pressure areas come available. In this light the exchange of results coming from applications of the originally suggested typical species composition (Candidate OSPAR Indicator BH1; OSPAR, 2018) would be highly valuable. Integration of the two methodologies making use of the original Typical Species Composition in case of clear single-pressure cases and the availability of reference situations and using those results to achieve reliable internal references for the BISI for application in multi-pressure or unknown quality status situations is suggested for application at regional/European scales (OSPAR, 2019).

It is obvious that not all species distributions are in line with geographical subdivisions as in use for management and assessment purposes (although it might be expected that those subdivisions have some ecological relevance as well). In case species compositions show large within region geographical differentiations where indicator species cannot be easily exchanged from sub region to sub region, or using higher taxonomic levels in the indicator does not solve the problem (or might impact sensitivity) it is advised to use sub region specific BISIs. An intermediate solution might be to use different internal reference levels for different sub regions to solve minor geographical differences in species compositions with potential impact on BISI results. Another option is to differentiate in threshold levels at sub regional scale. This might be a good solution for temporal management targets at small scale, but might be unwanted at large scale. For optimal clarity it is desirable that a threshold value for a good quality status is everywhere the same. It is suggested to test, apply and fine-tune the methodology that in the end it is clear that a BISI-value of 1 indicates a good quality status. For the time being a value of 0.5 considering uncertainties taken for granted in the methodology could serve as a threshold value for a sufficient good quality status. However, working with such a threshold should not be an invitation for disturbing activities in low pressure areas with high BISI scores. There the target should be that a significant decrease compared to the current situation is unwanted.

The other aspects of concern (low monitoring efforts or poor representativity) are not specific for the BISI, but problematic for any indicator. They will have an impact on reliability of outcomes of the BISI as well. Therefore it is advised to do power analyses for the monitoring programme in place related to the actual levels of occurrence of indicator species, on a regular basis, to indicate the realised power of the quality assessments. In case of poor representativity of the monitoring programme, it might be an option to focus quality assessments on one or a few (sufficient monitored) ecotopes. For several large areas of certain regions at the European scale it is more realistic that a quality assessment for a (small) part of the system is already a nice achievement, after which possible consequences for other parts of the system should be modelled or estimated based on expert judgement making use of minimal data available.

6.5 Data source and description of data

There are two types of datasets related to the current methodology. In the first place, there has to be a dataset of 'historic' data and/or data from reference areas at ecotope level in particular, on which the compiled reference levels used in the methodology will be based. Historic data are not directly involved in the calculation of IIS's, but are required for the selection of indicator species and assessment of the reference values. Then there is the dataset that will be evaluated. These data likely include data of some kind of T0 or initial state: A situation that is evaluated or to which other evaluation moments, possibly in the future, will be compared. Characteristics and essentials of both data sets will be described with examples from the Dutch North Sea case.

6.5.1 'Historic' data used to extract the reference levels

Although 'historic' might suggest that observations from decades to centuries ago might have been used; this is not the case. If available, such data are scarce, highly scattered (not covering all essential areas), often descriptive and difficult to match with current sampling methodologies. But most important, it is highly questionable if a historic reference level is a good reference level to use, as habitats currently present are modified by centuries of anthropogenic activities. If all pressures were taken away today, habitats and benthic communities would naturally not develop to pristine state (without any help), due to nowadays largely deviating habitat constitution and different species pools present. If certain parts would develop in the direction of some kind of pristine state, this would be a long-term process. The aim of the current methodology is to show quality improvements (or deterioration) on the short- and mid-term potentially as a result of changes in management and taken measures. Therefore a more realistic reference is used, based on current habitat constitution and present species pools, potentially showing first indications of quality improvements and/or deterioration. It is expected that the used compiled reference levels might reflect a realistic target in case the dominant pressures are reduced, but we are aware that future evaluations might indicate that certain reference occurrences should be adjusted on the mid-long term. BISI estimations from the past can easily be recalculated according to new reference levels if there are. With BISI v2 the derivation of the internal reference is more standardized according to the scheme presented in Figure 6.3.4.a, which includes the consideration of possible reference areas (ecotopes).

For example, the internal reference for the Dutch North Sea is based on large data sets on North Sea benthos covering the period 1984 till 2014 (with an exception of some older data for the 'Vlakte van de Raan') as described in Wijnhoven & Bos (2017). Initially (BISI v1), each of the available datasets were analysed for the areas to be evaluated. Maximum observed (year) average occurrences (either hit rate or abundances) were extracted and compared. The reference level consisted of the highest value for each species for a certain area if data coverage was expected to be sufficient (number of samples) and representative (spatial distribution and used methodology) for the area of investigation. Exceptions were made if highest occurrences were observed in recent years. In that case also the observations from 2015 were considered, maximum observed occurrences were either increased with the observed standard deviation or values were doubled, based on expert judgement. This procedure is with BISI v2 more standardized (and formalized) as indicated in Figure 6.3.4.a. Besides maximum occurrences, also standard deviations and patterns in occurrences in other data sets (neighbouring areas or suboptimal techniques) are considered, to identify whether peak occurrences might be missed due to under sampling in a certain period.

6.5.2 Essential data for evaluation

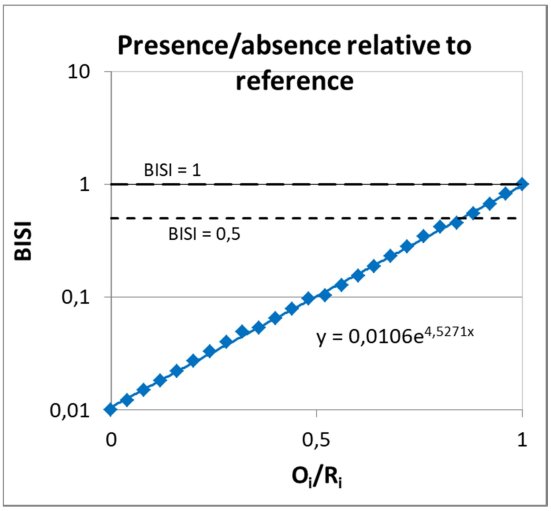
Basically any macrobenthic monitoring data should do, as long as some rules are considered:

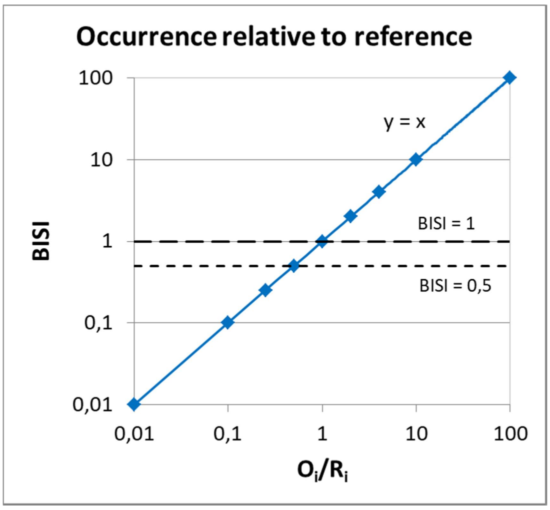
- Sampling methodologies should be suitable for the detection of benthic macrofauna and should fit to the selected indicator species in the index. Additionally methodologies of monitoring and derivation of reference occurrences should be comparable in terms of about the same surface, to a similar depth, with a similar mesh size at sampling, and identification to about the same taxonomic level. Certain species can potentially be monitored with various quite different techniques (e.g. boxcores and dredges), but with introduction of an alternative monitoring technique, new methodology specific reference values should be calculated if not available.
- Monitoring should be representative to give a good view of the quality status of the ecotopes of concern. Ideally sampling is random within the ecotope in a certain area, or a fixed monitoring grid (initial random selection) is continued. Such a monitoring can be random stratified over the (in surface area) dominant ecotopes. It should be determined if not an ecotope but a composite area (of various ecotopes) is the target for evaluation, or whether the monitoring is sufficient to give a representative view of the entire area (see next point).
- Monitoring efforts should be sufficient to give a representative and reliable view of the quality status. The minimum number of samples can be determined using power analyses for which a certain level of accuracy (what differences should be detectable within what timeframe) and a significance level is considered. It is expected that those are reasonable (realistic) numbers of samples as power analyses are part of indicator species selections as well so that only 'smart' indicator species are part of the general evaluations with BISI v2. If certain specific evaluations are of importance, it might be that the number of necessary samples is higher (or one should accept a lower power of these tests), as those evaluations include a smaller number of 'smart' species and can include less distinguishing 'indicator' species as well. There might be options to spread out monitoring over several campaigns or years and combine data as one moment/period to be evaluated to increase the sample size. Ideally those samples are than not from the same locations. Similarly evaluating a certain moment or period against a trend (based on several years), or comparing trends, might increase the power of the tests.

6.5.3 Conceptual testing of the methodology

Evaluation on basis of BISI leads to a quality score (BISI value) deviating between 0.01 and 100 as minimum and maximum values representing 100 times lower and 100 times higher than the internal reference occurrence (BISI = 1) of the indicator species. The index (consisting of a set of selected indicator species) responds however to the absence of indicator species, and the relative occurrence of those indicator species present.

Figure 6.5.3 Response curves of BISI.





a. Response of BISI-score when presence/absence of indicator species changes. The x-axe indicates the share of indicator species present relative to the number of species forming the internal reference. (An occurrence at reference levels is suggested when an indicator species is present). The BISI increases from the minimum score of 0.01 when no indicator species are observed to the reference level with a BISI-score of 1, when all indicator species are observed. The increase of the BISI as a function of O_i/R_i is in fact described by BISI = $\exp((1/S)*((\Sigma n_i*\ln(IV_i*(O_i/R_i)))/n_{avg}))$, with $IV_i = 1$ in this example where all indicator species have similar weight; but approaches BISI = $0.0106*\exp(4.5271*(O_i/R_i))$.

b. Response of BISI-score in case all indicator species are present. The graph shows an increase of all indicator species (in similar amounts relative to the reference) from an occurrence at a level 100 times lower than the reference occurrence ($O_i = 0.01*R_i$) via the reference occurrence for each of the indicator species ($O_i = R_i$) to an occurrence for all indicator species of 100 times the reference levels ($O_i = 100*R_i$). In this case with all indicator species present, BISI = O_i/R_i.

Basically, an occurrence 100 times lower than the reference occurrence of an indicator species is equal to being absent, whereas species transgressing the maximum occurrence of 100 times the reference get the maximum score similar to 100 times the reference.

To clarify the response of BISI to differences in observations the presence and absence of species, and the impact on the BISI score, is shown separately (Fig. 6.5.3.a) from the effect of changes in occurrences of species (Fig. 6.5.3.b). In practice the BISI-score will be the result of the combination of both aspects deviating, where species occurrences differ independently. Additionally, differences in indicator values of individual species might result in a slightly larger impact of differences in occurrence of the one species on the resulting BISI score than from the other.

The graphs indicate that the presence or return of each additional indicator species is not of about similar importance but according to a logarithmic function (Fig. 6.5.3.a), which makes that the difference on the BISI score of one species missing is higher when the indicator species community is almost complete, than in case any indicator species are missing. The impact of abundance of indicator species on the observed quality status (BISI score) is a linear response (Fig. 6.5.3.b) which makes that a doubling of the numbers leads to a doubling in the quality status, independent whether few or many specimens are present. However, to a certain level, as occurrence is truncated at 100 times the reference value (higher or lower), so that BISI values can never transgress beyond 100 or sink below 0.01. The combination of these two responses means that the presence or absence of indicator species is more important towards the quality status than the relative occurrence of these indicator species especially when most indicator species are present. When only a few indicator species are present, their relative occurrence is more important. When all indicator species are present, the quality status increases equal to the increase of average relative occurrences of the indicator species. I.e. indicator species transgressing set reference abundances still have an (additional) positive impact on the quality score. It is expected that the risk of indicator species becoming nuisance species is small; otherwise they were not selected as indicator species in the first place, so that their increase indeed might be a sign of quality improvement. When however such an increase in abundances might have ecological negative impacts it is expected that this will be reflected in the occurrence of several other indicator species, in the end leading to a lower BISI score.

7 References background information on methodology

For details and background information, specifically on the development of BISI v1 (on which v2 proceeds), see:

- Wijnhoven, S., Bos, O.G. (2017). Benthic Indicator Species Index (BISI): Development process and description of the National Benthos Indicator North Sea including a protocol for application. Ecoauthor Report Series 2017 - 02, Heinkenszand, the Netherlands.

The report includes a BISI Assessment Tool (Annex 2): V260917, is the assessment tool based on BISI v1 including indicator species lists references and methodologies as applied for the Dutch North Sea. For details and results of the first application and a recent historic analyses of the quality developments based on BISI (v1) for the areas of evaluation as identified in the Dutch North Sea, see:

- Wijnhoven, S. (2018). T0 beoordeling kwaliteitstoestand NCP op basis van de Benthische Indicator Soorten Index (BISI). Toestand en ontwikkelingen van benthische habitats en KRM gebieden op de Noordzee in en voorafgaand aan 2015. Rapport Ecoauthor & Wageningen Marine Research. Ecoauthor Report Series 2018 - 01, Heinkenszand, the Netherlands (in Dutch).

The adjustments made to the methodology and presented here (BISI v2) are implemented in the BISI Assessment Tool v311219 for the areas and habitats of evaluation in the Dutch North Sea. An exception are the indicator species selections for the areas and habitats of evaluation, that are not adjusted according to the, with v2 introduced, decision scheme, but that are consolidated as originally constructed with BISI v1. The BISI Assessment Tool v311219 may function as an extensive example of application of BISI v2 towards application in new areas:

- Wijnhoven (2019a). Assessment tool: 'Benthic Indicator Species Index (BISI)': Application of BISI v2 in the Dutch North Sea with consolidation of earlier identified references. BISI assessment tool in Excel, v311219.

New applications are the construction of BISIs for evaluation of marine (and estuarine) Habitat Directive habitat types and HD areas, situated outside the subtidal North Sea as present in the Netherlands. Those assessment tools are completely constructed according to the current methodology, as described in detail in:

- Wijnhoven, S. & Van Avesaath, P.H. (2019). Benthische Indicator Soorten Index (BISI) voor mariene habitattypen in Natura 2000-gebieden. Uitwerking beoordelingsmethodiek inclusief monitoringvoorstel voor mariene habitattypen van de Habitatrichtlijn gelegen in de Deltawateren, het Waddenzeegebied en de kustzone van de Noordzee. Ecoauthor Report Series 2019 - 03, Heinkenszand, the Netherlands (in Dutch).

Presented with an application tool (including a brief manual) in English:

- Wijnhoven, S (2019b). Assessment tool 'Benthic Indicator Species Index (BISI)': Application of BISI v2 for marine Habitat Directive habitat types of the Dutch 'Delta-waters', the Wadden Sea and the coastal zone of the North Sea. BISI assessment tool in Excel, v070120.

The current protocol is specifically developed for generic application which allows application in the international context, amongst others with regards to the MSFD, HD and OSPAR context. As a first step, for testing and application, an assessment tool is developed to cover the southern North Sea and small part of the northern North Sea subdivided into 6 subtidal soft sediment broad habitat types:

- Wijnhoven (2019c). Assessment tool: 'Benthic Indicator Species Index (BISI)': Application of BISI v2 in soft sediment habitats of OSPAR region II (Greater North Sea region). BISI assessment tool in Excel, v311219.

All products (reports, protocols, application tools and evaluations) will be made available via the Ecoauthor website: www.ecoauthor.net, where a specific page on BISI will be created. Search term for information: http://ecoauthor.net/?tag=bisi.

8 Strengths and weaknesses of data

8.1 Strengths

Besides that (general) changes in the quality status can be detected for the different areas, ecotopes and habitats under investigation, the indicator gives insight in the causes and effects of observed changes.

Even if changes are not significant (yet), a series of evaluations might indicate whether developments seem to move into the direction of a good ecological status or might indicate whether quality improvement or deterioration can be expected in the near future.

The focussing of the methodology and the monitoring programme on specific management measures gives insight in the local effectiveness and the influence of measures on larger scales, the relative importance of different pressures on the current quality status, and the potential of (additional) measures in the future.

As the methodology makes use of a reference based on real observations of the last three decades, a realistic reference is ensured, that is within reach with effective management. This is at the moment at least the case for the Dutch marine and estuarine waters including the North Sea and the specific management areas situated in these waters. Although the methodology is specifically developed to evaluate change in quality status in time or between different treated areas making use of a fixed monitoring design, a comparison of the T0 quality status relative to the realistic reference is expected to be reliable concerning the relative importance of causes and effects of differences in quality status.

The BISI indicator makes use of a range of indicator species with deviating indicator value related to different pressures. This makes the methodology robust, so that it is not susceptible to coincidental changes in occurrences of singular species. Moreover, the methodology appears to be robust enough that an occasional missing of samples does not lead to another interpretation of the quality status (it can lead to a decrease of importance of especially less common or 'highly variable' species in the assessment of the quality status). It has been found that even an evaluation based on one of the two dominant sampling methodologies (evaluation only based on boxcore samples or only based on dredge samples) generally leads to comparable results for quality status assessments (i.e. Wijnhoven, 2018). Such a reduction of the monitoring efforts and the number of indicator species involved does however impact the likelihood of finding reliable results for the specific indices indicating causes and effects.

With BISI v2 additional species might be added to specific evaluation, although they do not reach the criteria set for the general quality evaluation. Specific power analyses should indicate whether the resulting specific indicator is more sensitive with or without the inclusion of certain potential indicator species.

By taking ecotopes and specific BISIs at ecotope level, as the basis for the methodology, the indicator is in case of sufficient sampling, also capable of indicating (indicator species) community changes as a result of changes in the ecotope composition. Such changes can either be the result of natural processes or human induced. This, and the natural or preferred occurrence of certain ecotope types in areas of concern, will determine whether changes can be considered a decrease or an improvement of the quality status. If such changes in habitat constitution are a target (of management measures) on beforehand, the internal reference can be constructed considering the preferred area ratio distribution. It is likely that the loss of highly valuable ecotope (either whether it is potentially most species diverse, provides high benthic biomass or whether it is a scarce habitat and is therefore important as it can contain very specific additional species) will also lead to a lower BISI score and *vice versa*.

8.2 Weaknesses

As the methodology is in the first place developed to detect changes in the quality status between the T0 and future evaluations, the accurateness of the internal (realistic) reference is something that has to be confirmed in the near future by the results of the coming monitoring years (according to the national benthos monitoring programme). It is very well possible that (as expected) the internal reference in use now is a reliable reference level for a realistic GES, in terms of what can be reached naturally on the mid-term if dominant pressures are diminished. This is however something that should be tested by using the evaluation tool for future evaluation, by focussing on areas with effective management measures, possibly combining sample locations with detailed pressure mapping, and/or application of the methodology in areas outside the Dutch continental zone (NCP). It is expected that the identification of potential causes and effects of observed quality differences is reliable (see also 8.1 strengths). It is however not completely certain at present whether the internal reference should be slightly adjusted, and more important, if this is more the case in certain areas to be evaluated than in others. The reliability of the internal reference is likely dependent of the monitoring efforts in the past (that show spatial differences).

With BISI v2 the derivation of the internal reference is more standardized making evaluations of different areas more comparable, although certain aspects as monitoring efforts, habitat heterogeneity at a level hidden by the aggregated ecotopes and present species pools will to a certain extent have some impact on evaluation results. Additionally a broader application in various regions, including possible identification of reference areas, might give opportunities for improvement of the comparability of evaluations. Therefore recalculations of internal references as a calibration measure are suggested for areas for which after installation of a BISI methodology a series of monitoring campaigns has come available.

A weakness related to data availability is that there is variability in the methodologies used for sampling, sorting and identification. It is known that the indicator is to a certain extent susceptible to the use of different methodologies. It is therefore at the moment at the regional/European scale still difficult to relate observed differences in BISI scores to possible quality status differences or differences in sampling methodologies, taxonomic identification and monitoring strategies. Common testing and application in international case studies should clarify this. Additional, as it is foreseen that these differences will also prevail at least between countries in the future, it is highly advised to install a joint monitoring that can function as a baseline. Herewith national findings can be related, comparted and put in perspective (this is valuable for most if not for all benthic indicators).

Low sampling efforts and/or low representability of monitoring designs can result in deviating results, but also this is probably the case for most indicators. A weakness of the methodology might be that it is possible to calculate a BISI based on a few samples. It is therefore advised to always take the power (and representativeness of the sampling strategy) into account.

Another uncertainty is related to the debate around indicator value of some of the species. It is a fact that not all ecological aspects are known even for rather common marine species. It is expected that possible new insights in the ecology of species and their indicator value, will have minimal impact on evaluation results as the number of species under debate is kept to a minimum and the methodology is based on a large number of species to reduce possible impacts. With BISI v2, and the application within the frame of OSPAR, it is foreseen to align the characterization and qualification of potential indicator species by using the same sources and central storing of indicator characterisations of species per ecotope (possibly integrated in existing platforms).

9 Further work required

First of all the methodology will benefit from application the coming years in a variety of areas under different circumstances, to identify possible weaknesses. At present the T0 evaluation has been performed, including recent historic analyses of quality developments for the Dutch North Sea areas on basis of BISI v1. As the methodology (with BISI v1) is actually developed to detect changes in the quality status instead of exactly defining the quality status at a certain moment, the first real test on a full-scale specifically designed monitoring programme will be on basis of the 2018 monitoring data.

At present also assessment tools for HD areas and habitat types outside the subtidal North Sea have been developed on basis of v2. During the developmental phase some testing has been performed for (habitats) of the Western Scheldt, the Eastern Scheldt, the Wadden Sea (including Ems-Dollard) and the intertidal North Sea coast. The methodology could benefit from some further testing in real applications, like analyses of historic developments in benthic quality at the level of basins, including new areas with the same habitat type (e.g. lake Veerse Meer with the BISI for H1160). Smaller scale applications in test cases with high data density related to substrate suppletion and comparison with (other) multi-variate analyses is foreseen with the BISI for H1110b. The methodology could benefit from application in test cases around management zones comparing developments in different zones, like application around offshore wind farms and areas with fisheries restrictions. Other applications of interest that can improve or provide new insights are application around construction and restoration of foreshore defences including construction of elements to support the recovery of soft sediment habitat types afterwards in different designs (in the Eastern Scheldt). Investigating options for additional specific evaluations, like the relation of benthic quality to non-indigenous species impacts, would be valuable as well.

Additionally assessment tools for application at (sub)regional level have been developed and first testing and application has been performed with regards to the OSPAR region II and the southern North Sea (and small part northern North Sea as well) in particular. Here challenges seem to be particularly related to scale and possible geographic differences in species occurrences and therefore BISI results when using a specific indicator. However, other challenges like differences in monitoring methodologies, spatial and representative coverage, and information on management measures (including efficiency) and insight in pressure distribution, are even much larger. Here the methodology development could benefit from application in joint international test cases with reasonable data availability at a slightly smaller scale, like around the Dogger Bank and Kattegat. Indicator development could highly benefit from the installation of joint monitoring programmes and further standardization of monitoring at the regional scale, for which opportunities and options are investigated at the moment (Van Hoey & Wittoeck, 2019; Vina-Herbon & Guérin, 2019). This is not only of importance for indicator development, but also essential to achieve comparability among quality assessments and reporting at the regional scale.

The methodology will additionally benefit from evaluation based on detailed pressure maps, and the possible identification of low pressure regions for validation and/or alternative derivation opportunities for the internal reference as formalized in BISI v2. This especially in comparison and/or combined with the application of the original Typical Species Composition indicator (OSPAR, 2018) as suggested with the presentation of a draft CEMP for an integrated BH1 (combining Typical Species Composition and BISI; OSPAR, 2019). Therefore application outside OSPAR region II (e.g. application in regions III and IV) might be beneficiary as well.

The evaluation tool is developed as a living methodology, which allows adjustment of used selections of indicator species, indicator values and reference values according to new insights. Although in that case recalculation of earlier evaluations will be needed, those can be realized with limited efforts. The methodology is robust enough so that these adjustments will not dramatically change earlier outcomes.

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