Protocol Benthic Indicator Species Index (BISI):

Protocol BISI for generic application (BISI v3). Version v170424.

– Wijnhoven (2023)

The protocol describes the Benthic Indicator Species Index (BISI) for generic application: BISI v3. The BISI is a benthic habitat quality assessment indicator that compares the combined occurrence of indicator species to a realistic good quality status reference indicator species community. Basically developed at the level of ecotopes, BISIs can be constructed for any composite area by following the ecotope surface distribution. The BISI provides a general quality status value on a scale from 0.01 to 1 (as values >1 should be considered equal to BISI=1) at which BISI=1 represents a good quality equal to the realistic reference status. In general quality status assessments all (selected) indicator species are of equal importance. Related specific quality assessments particularly focus on specific aspects of quality, by focussing solely on indicator species sensitive for specific pressures or environmental changes, and on quality of ecological functioning aspects solely focussing on species providing those functions. In specific BISI assessments, those specifically selected indicator species can have a different weight (species specific indicator value) in the calculation which equals a value between 0 and 1 for none-indicative to very indicative species towards the specific assessment.

The protocol is accompanied by Assessment Tools that describe the application in detail and that can be used for application of the BISI in practice by, in principle, completing observations (in desired format) and selection of relevant areas or habitats, indicator species depending on monitoring efforts, assessment objectives and observation methods:

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

- *Wijnhoven (2023a). Assessment tool: 'Benthic Indicator Species Index (BISI)': Application of BISI v3 in the Dutch North Sea with consolidation of earlier identified references.* v021023.
- *Wijnhoven (2023b). Assessment tool 'Benthic Indicator Species Index (BISI)': Application of BISI v3 for marine Habitat Directive habitat types of the Dutch 'Delta-waters', the Wadden Sea and the coastal zone of the North Sea.* v061023.

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

- *Wijnhoven (2023c). Assessment tool: 'Benthic Indicator Species Index (BISI)': Application of BISI v3 in soft sediment habitats of OSPAR region II (Greater North Sea region).* v031023.

Although former BISI versions can still be applied and only deviation in absolute values and differences in BISI scores are expected (not in developmental patterns and whether results indicate quality improvement or decrease), it is proposed to use BISI v3 for quality assessment, and in case of recurrent quality assessment replace former findings by results based on BISI v3. BISI v1 and v2 (that have been launched and used in benthic habitat quality assessments before) deviate from BISI v3 in the way that:

- *focus of BISI v1 was on quality assessment of defined areas with consolidated monitoring programmes whereas with BISI v3 besides consolidation of earlier identified area specific references, ecotopes form the basis for derivation of references, which allows construction of references for any composite area as well;*
- *compared to BISI v1, (standardized) rules for indicator species selection (and the construction of BISIs for other ecotopes/regions) are part of the methodology of BISI v3;*
- *adjustment of the BISI formula in BISI v3 compared to BISI v2 by placing the species-specific indicator value IV_i (calculated as the standard indicator value divided by the average value of the included indicator species $=iv_i/iv_{avg}$) outside the log-term in the formula;*
- *halving of iv_i in BISI v3 compared to BISI v2 in case $iv_i<1$ (to increase importance of most indicative/sensitive species);*

and additionally some issues with calculation of the pooled standard deviation have been solved in BISI v3.

Recent adaptations allow BISI development and application in basically any area or region with sufficient data availability.

This protocol should be cited as:

Wijnhoven, S. (2023). Protocol Benthic Indicator Species Index (BISI): Protocol BISI for generic application (BISI v3). Version v170424. Ecoauthor Report Series 2023 - 01, Heinkenszand, the Netherlands.

(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)

$$BISI = exp\left(\left(\frac{1}{S}\right) * \sum\left(IV_i * \ln\left(\frac{O_i}{R_i}\right)\right)\right)$$

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

The current BISI v3 version was amongst others developed in cooperation and discussion with L. Soldaat and M. Poot[1] (Statistics Netherlands; CBS) and in discussion with P. Herman (Deltares/TU Delft) and V. Escaravage (Wageningen Marine Research; WMR), as part of contracts commissioned by the Dutch Ministry of Agriculture, Nature and Food Quality (Min LNV) with special thanks to J. Vonk as the commissioner in particular. Contributors to former versions are: P. van Avesaath (AMAECON[2]) who assisted in developing the methodology for BISI v2, L. Soldaat, M. Poot and R. Beij (CBS) who commented on BISI v2. The first version of the BISI (v1) was developed parallel to a number of project meetings to discuss ideas and progress in cooperation with and commissioned by A.-M. Svoboda (Min LNV, formerly Min EZ), S. Stuijfzand (Ministry of Infrastructure and Water Management; Min IenW, formerly Min IenM) and J. Vonk (Min LNV). Special thanks to O. Bos (WMR) being the editor of protocol v1 and to all experts[3] 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 (WMR), O. Beauchard, V. Escaravage (Netherlands Institute for Sea

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Research), E. Verduin (Eurofins), A. van Strien (CBS). The BISI has been presented and discussed in the OSPAR Benthic Habitats Expert Group (OBHEG) and in the EU Marine Strategy Framework Directive Common Implementation Strategy Technical Group Seabed (TG-Seabed) as well.

3 Key message

The Benthic Indicator Species Index (BISI) is an indicator making use of benthic community observations (benthos data) to assess benthic habitat quality status, sea floor integrity and aspects of ecological functioning. The BISI compares the combined occurrence (densities or presence/absence ratio) of area or habitat specific indicator species communities with predefined realistic reference occurrence under good quality conditions resulting in a relative quality score (BISI score between 0.01 and 1, considering a BISI>1 as equal to the good quality reference state of BISI=1).

The BISI provides an assessment of the general quality status of an ecotope (habitat) or aggregated ecotope area and potentially several specific assessments to identify possible causes and effects of the observed quality status (and their importance), based on subsets of indicator species. Herewith the BISI closes the gap between traditional observation based benthic habitat quality indicators (like diversity indices and total community properties) and pressure based spatially explicit impact modelling by indicating possible causes of observed quality status and/or developments. Additionally the BISI provides the opportunities (via specific assessments) to estimate the consequences of observed quality status and developments towards ecological functioning aspects. A selection of specific assessments typically includes, but is not limited to, the severity of potential pressure impacts of physical disturbance (including differentiation in recurrent/frequent or high impact/deeper (>2 cm) into the sea floor penetrating disturbances of for instance fisheries or aggregate extracting activities), ecological disturbance (like impact from organic enrichment, hypoxia and/or toxic substances) or changes in environmental conditions (e.g. hydrographical changes, changes in sediment composition). Specific assessments with regards to consequences towards ecological functioning could include assessment of structure function, food web function and biological activation function, respectively focussing on the occurrence of species important in providing tertiary structures and improved habitat complexity/variety, important as food items for species of higher tropic levels, or bioturbating and bio irrigating species.

Selection of indicator species and derivation of reference levels is part of the methodology with BISI v3 taking place at the level of (high aggregation) ecotopes (e.g. MSFD broad habitat types according to EUSeaMap 2021; Vasquez et al., 2021) or other ecotope classifications with clearly to distinguish benthic communities). It is suggested to consolidate reference indicator species communities (i.e. indicator species selections taking monitoring methodology and efforts into account, and reference occurrences) at the level of ecotopes within (sub) regions for future assessments (as much as possible). BISIs for composite areas can be constructed and used at different spatial scales by combining ecotope-specific BISIs on basis of ecotope surface distribution, or by averaging BISI scores of separate ecotopes taking surface distribution into account. The first to define a BISI for consolidated assessment areas in need of recurrent quality assessments (in line with the use of consolidated reference indicator species communities as defined for specific areas within the Dutch part of the North Sea during development and application phase of BISI v1; Wijnhoven & Bos, 2017). The second to allow more flexible application in dynamic environments or in case of absence of consolidated monitoring programmes making use of opportunities for assessments based on occasional just partially representative (without full spatial area coverage) project data.

Indicator species are selected according to a standardized derivation scheme 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. The BISI makes use of realistic indicator species references as the potential possibility to be present or the opportunity to return (suitable indicator species are part of the current species pools) is a prerequisite for eligibility as an indicator species. 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 considering natural variation. 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 to obtain reference occurrences of selected indicator species.

Functioning at different spatial scales, the BISI can be the tool to assess quality status or quality developments at (sub) regional, national, assessment unit, marine reporting unit or other subunit scale, including Natura2000 areas and habitat (sub) types, Marine Protected Areas (MPAs) and specific management areas. The 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. Specifically developed for benthic habitat quality status assessment for marine waters based on benthos; application in other systems and types of habitats and inclusion of other types of biota as indicators and alternative observation techniques and/or observation data is possible.

4 Definitions

Area specific index – Index designed/compiled for a specific area to be assessed. Here used as the area specific BISI; indicating that each

	area of assessment has its BISI, where indicator species composition with their reference occurrences depends on the constitution of the area. The compilation of an area specific BISI is with v3 more standardized (compared to v1), as it builds on standard references/BISIs for ecotopes (BHTs) that are combined/averaged to an area specific BISI according to the ecotope surface distribution.
BHT	- Broad Habitat Type, generally referring to the EU MSFD Broad Habitat Types according to Vasquez et al. 2012 (EU-Seamap2021) and Raicevich et al. (2024). The accompanied Assessment Tool 'Application of BISI v3 in soft sediment habitats of OSPAR region II (Greater North Sea region) (Wijnhoven, 2023c) refers to BHTs which in some cases are combinations of EU MSFD BHTs (i.e. combining infralittoral and circalittoral variants).
BISI	- Benthic Indicator Species Index. Benthic community observation-based indicator to assess benthic habitat quality status and quality developments as presented and elaborated in this protocol.
BISI score	- Score (also indicated as BISI value) that reflects the benthic habitat quality status on a scale from 0.01 to 1 (from very low quality status to good quality status equal to the realistic good quality reference). In theory the BISI score can be up to BISI=100; in practice the value will however seldomly be above BISI=1, and it is suggested to consider a value of BISI>1 as being equal to the realistic good quality reference of BISI=1. Taking uncertainty, realistic monitoring efforts and the qualitative description of a threshold value according to Raicevich et al. (2024) into account; a BISI value of 0.5 might be a suitable threshold value for good quality status with regards to the MSFD for the time being.
BISI v1	- Original version of the Benthic Indicator Species Index, specifically developed for assessment of fixed areas (HD - and sea floor protection areas) and habitats of the Dutch North Sea. V1 is especially deviating from BISI v3 in the way references are constructed area specific. In v2 (and v3) compared to v1 an adjustment of the transformation and back-transformation has

taken place so that results in analyses of quality developments (trends) are less skewed[4].

BISI v2 - The second (updated) version of the Benthic Indicator Species Index, especially deviating from BISI v1 in the way that references are defined ecotope specific. A reference for an area to be evaluated is compiled, considering the ecotope surface distribution, from the standard references of the underlying ecotopes. In v2 the transformation and back-transformation leading to more skewed results in analyses of quality developments (trends) was adjusted. Other deviations were a further standardization of indicator species selection, reference level derivation and corrections in the methodology of calculating pooled standard deviations associated with the BISI.

BISI v3 - Current latest version of the Benthic Indicator Species Index. Compared to BISI v2 the formula to calculate a BISI score has been adjusted by placing the Indicator Value (IV_i; an indicator species specific weight factor) outside the log-term. Additionally the IV_i values have been reduced to half of the original value in case the IV_i was less than 1. Both adjustments lead to more emphasis on the most indicative species in the assessment results and are particularly meant to achieve more distinction between the specific assessments to improve detectability of possible pressures leading to the observed quality status or - development as indicated by the BISI and/or indicating possible consequences of observed quality status or - developments with regards to ecological functioning. As the formula has been changed this has consequences for the calculation of the pooled standard deviation as well; which is adjusted accordingly.

Ecological disturbance – Disturbance of habitat and benthic communities in particular by presence of toxic substances, pollutants, organic enrichment and hypoxia. 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

[4] Transformation in v1 was according to $^{10}\log$ (instead of a natural logarithm in v2 and v3) while back-transformation consisted of the inverse natural logarithm. Results of the each of the BISI versions, point in the same direction, but there is a difference in the steepness of trends.

based on any ecotope classification system, preferably widely accepted classifications like EUNIS (e.g. EU MSFD Broad Habitat Types) and (particularly for transitional waters in the Netherlands) 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 3 (indicated as MSFD Broad Habitat Types) or aggregations of those are suggested for application of the BISI at the European (sub) 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).

Indicator Value – Indicated with IV_i, the indicator value is the species specific standard 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 assessment ($IV_i=iv_i/iv_{avg}$).

Realistic reference – Also indicated as 'internal reference'; the reference community of indicator species in reference occurrences (abundance or presence/absence ratio) expected to be present in case of absence of or low pressures situations, reflecting good quality condition of the benthic habitats, used in the BISI to calculate the relative occurrence of indicator species; observation divided by the reference occurrence. The internal reference is indicated as a 'realistic' reference as only indicator species with opportunities to return (present in regional species pools) are considered and reference occurrences take into consideration nowadays environmental conditions and therefore likely deviate from pristine reference conditions and communities. The internal references used in BISI are derivate from maximum potential observations taking natural fluctuations into account in case no data from reference areas are available. The reference BISI score per definition equals 1.

MSFD - Marine Strategy Framework Directive.

MSFD broad habitats – Habitat types specifically distinguished in the current EUNIS classifications (EUSeaMap2021; Vasquez, 2021), suggested by the EU as the working and assessment units with regards to MSFD reporting (Raicevich et al., 2024).

Occurrence - Densities or presence/absence ratio (potentially also biomass) used as observation and reference data reflecting abundance of indicator species.

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 (densities or presence/absence ratio) is used to allow standardized (relative) comparison of abundance observation data for indicator species. The BISI makes use of a realistic (internal) reference, which deviates from the pristine reference situation.

Smart species – 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 BISI methodology a realistic effort is expected to be a maximum of 60 samples (for an area or habitat of concern with the relevant observation technique) necessary to detect 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 (insensitive; not included in a representative specific assessment) and 1 = a very good indicator (sensitive species) for the specific assessment. IVi is calculated by dividing the species specific standard indicator value iv_i by the average indicator value for all indicator species included in the specific assessment iv_{avg}.

Threshold value – A value that should be reached to indicate a good quality status; at the moment there is no established quality threshold value yet for MSFD quality assessment under D6C5 (Condition of the benthic habitats) and D6C3 (Adverse effects of physical disturbance on benthic habitats), and neither under OSPAR. Nevertheless a potential threshold value for D6C5 has been described qualitatively (Raicevich et al., 2024), so that it can be estimated that a potential threshold value distinguishing good quality habitat from habitat suffering adverse effects, could be around BISI=0.5.

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 Sentinels of Species (SoS) benthic habitat quality indicator BH1 makes use of 'typical species' defined by high intra-habitat similarity and frequent occurrence under reference conditions (OSPAR, 2021; Serrano et al., 2022)

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.

(In theory BISI could potentially be developed for other ecosystem components/communities as well in case of sufficient and representative monitoring data).

5.2 MSFD Descriptor

D6 Sea floor integrity:

- D6C5 'The extent of adverse effects from anthropogenic pressures on the condition of the habitat type, including alteration to its biotic and abiotic structure and its functions (e.g. its typical species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species), does not exceed a specified proportion of the natural extent of the habitat type in the assessment area'.
- D6C3 'Spatial extent of each habitat type which is adversely affected, through change in its biotic and abiotic structure and its functions (e.g. through changes in species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species), by physical

disturbance. Member States shall establish threshold values for the adverse effects of physical disturbance, through regional or sub regional cooperation.' (European Commission, 2017).

The BISI directly provides a benthic habitat quality status assessment based on benthic community composition including (and particularly focussing on) above mentioned typical – and sensitive species and species with key functions, as the resultant of all prevailing disturbances and environmental changes (D6C5). Specific assessments clarify which particular sensitive species or species with key functions lack or are present in reduced abundance due to what most likely reason (those pressures leading to impact as reflected in the quality status). One of the pressures that might result in impact that is (currently) subject of a specific assessment is 'physical disturbance' optionally further specified into 'recurrent' and 'more intense' physical disturbance, so that the BISI (potentially) also provides input for D6C3.

EU MSFD guidance for D6 assessment indicates that D6C5 assessments should preferably include several benthic community observation based indicators and might benefit from pressure based impact assessment for inter- and extrapolation purposes (also considering the extent of habitat loss; D6C4) (European Commission, 2022; Raicevich et al., 2024). As an example the Dutch Marine Strategy (part 1) combines the results of BISI application with OSPAR BH2b (Relative Margalef diversity indicator) and BEQI2 (WFD/OSPAR BH2a) application to come to a D6C5 assessment, whereas D6C3 is covered by OSPAR BH3 (Extent of physical disturbance to benthic habitats impact modelling) application (Min IenW et al., 2024).

5.3 Status

BISI is an indicator with the first version developed in the Netherlands in 2016/2017 for the Ministry of Economic Affairs (Wijnhoven & Bos, 2017). In the meanwhile the BISI has been used for benthic habitat quality assessment, including identification of recent historic trends and trend breaks, for the areas and habitats of the Dutch part of the North Sea within the frame of the MSFD (e.g. Wijnhoven, 2018; Wijnhoven, 2023; respectively making use of BISI v2 and v3), published as the Dutch Marine Strategy (part 1) (Min IenW & Min LNV, 2018; Min IenW, 2024). The indicator has been used for benthic habitat quality assessment for Habitats Directive marine habitat types H1110 and H1170 in Article 17 reporting (Janssen et al., 2020; making use of BISI v2). The indicator has been proposed and discussed as a potential candidate OSPAR indicator for Benthic Habitats quality assessment within the OSPAR Benthic Habitats Expert Group, where it was agreed that it could be an additional BH indicator, fulfilling a complementary role to the current set of indicators forming the BH assessment framework. In 2024 the indicator will be forwarded for a pilot assessment for region II (Greater North Sea region). Additionally the indicator has been applied to identify and analyse potential short term effects of sublittoral pilot sand suppletion (Van Hal et al., 2021; Wijnhoven, 2021; making use of BISI v2).

5.4 Indicator type

State indicator, however, by identifying most likely pressure types with impact resulting in the observed quality status or quality developments, filling the gap between benthic community observation based quality assessment (traditional quality indicators) and pressure based impact modelling.

6 Indicator description

6.1 Indicator goals/objectives

The Benthic Indicator Species Index (BISI) aims to assess the benthic habitat quality status, quality status developments and quality status differences for defined spatial areas, habitats or ecotopes.

In addition, the BISI aims to identify the importance of different potential pressures with potential impact leading to the observed quality status or observed changes in quality status. Simultaneously the potential effect of observed (reduced) quality status or changes in quality status on aspects of ecological functioning are identified. (See amongst others Tables 6.3.3.a and 6.3.3.b).

An area/habitat specific BISI consists of an (overall) general quality status index comparing benthic community observations with a (good quality) reference indicator species community. A general quality status assessment is optionally combined with several specific quality assessments that are based on weighted subsets/selections of indicator species with specific characteristics and/or traits. Selections of species with specific sensitivities are therefore indicative for specific relative pressure levels via impact leading to the observed quality status. Selections of species with specific characteristics for which higher abundances or aggregations are of added value towards specific ecosystem functions, indicate the level of ecological functioning with regards to that aspect.

Although, in case of time series of sufficient and representative data for periods with differences in pressure levels (preferably including low pressure situations), area specific BISIs can be constructed (as has been done with BISI v1; Wijnhoven & Bos, 2017). Since v2, BISIs are developed at the level of ecotopes (or broad habitat types) and are combined following ecotope surface distribution to construct the reference for (composite) areas to be assessed. Herewith basically each random area can be assessed with the BISI in a standardized way, given that BISIs are available for underlying major ecotopes. Considering the presence of sufficient and representative monitoring for an area to be assessed, the BISI can also identify possible changes or developments in ecotope/habitat constitution. (Such changes can be the result of natural developments or due to anthropogenic impact). The BISI intends to be a well-documented, reproducible and efficient assessment method that includes a protocol and (ready to use) assessment tools.

BISI v1 was specifically geared for assessment 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. Both approaches of assessment (already developed references for indicator species communities at the level of identified areas and references of ecotopes/habitats as the bases for compiled areas) have been consolidated into BISI v3 where way of calculation (formula) and relative importance of indicator values have been adjusted.

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 intensity (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[5] in their potential abundances reflect the quality status in case 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

[5] 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.

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 realistic (moderate numbers of samples) monitoring efforts (so that species that are present in good quality reference abundances are not likely missed by chance due to unsuitable monitoring techniques or insufficient efforts).

With BISI the combined occurrence of an indicator species community (indicative for the general benthic habitat quality status) compared to the occurrence under good quality reference conditions, is assessed. BISIs are habitat (or ecotope) specific, where reference indicator species communities reflect the habitat constitution of the area of concern to be assessed. Additionally, further differentiation in specific assessments based on those 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. In those specific assessments, most indicative/sensitive/'important towards an ecological function' species are given a higher weight (called 'indicator value') in the BISI calculations, whereas species that are to some extent indicative/sensitive/important receive a lower indicator value. Comparing the quality status based on BISI for different years allows assessment 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 (indicator species community) at a certain moment of assessment is compared with defined reference values for that selection of indicator species. The methodology consists of the calculation of the weighted (species - and assessment specific indicator values) geometric mean (i.e. ln-transformed) of observation to reference ratios. Testing occurs against a (fixed) good quality status reference compiled in a standardized way for ecotopes or habitats. With the BISI for generic application, BISIs for ecotopes and habitats form the basis for assessments of any (random) area or habitat (consisting of and characterised by those ecotopes or habitats). BISIs (i.e. indicator species lists with reference occurrences) are compiled from the individual (most important) ecotope references for those composite areas in a standardized way following ecotope surface distribution. BISIs are sampling methodology specific, although observations coming from different observation techniques can be combined in one BISI. Examples of areas of application are areas with a certain protection status, management regime or user function including Habitats Directive habitat types (covering several ecotopes).

At present, (ready to use) BISI assessment tools according to BISI v3 are available for:

- Areas and habitats of the Dutch part of the North Sea:
  The assessment tool includes BISIs for management areas (including Habitats Directive (HD) areas and MSFD areas[6]), MSFD Broad Habitat Types (BHTs; Vasquez et al., 2021; Raicevich et al., 2024) (or aggregations of those BHTs that are similar to EUNIS level 3 habitats) and marine HD habitat (sub) types.
  BISIs are specifically compiled for:
    - MPAs: Dogger Bank (Doggersbank), Cleaver Bank (Klaverbank), Central Oystergrounds (Centrale Oestergronden), Frisian Front (Friese Front), Brown Bank (Bruine Bank), North Sea Coastal Zone (Noordzeekustzone), 'Pro Delta(ic plain)' (Voordelta), 'Plain of the Raan' (Vlakte van de Raan); including applicability in related 'closed areas for sea floor disturbing fisheries[7]' and 'open areas for comparison' where relevant.
    - BHTs: 'Offshore circalittoral coarse sediment' (including small part of mixed sediment habitat), 'Offshore circalittoral sand', 'Offshore circalittoral mud', 'Circalittoral coarse sediment' (including small part of mixed sediment habitat), 'Circalittoral sand', 'Circalittoral mud'; the indicated 'circalittoral habitat types' are in fact combined circalittoral and infralittoral habitats.
    - HD habitat (sub) types: H1170 'Reefs', H1110b 'Permanently flooded sandbanks in the coastal zone', H1110c 'Permanently flooded sandbanks in the offshore'.

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[6] Both can be considered Marine Protected Areas (MPAs) although specific measures to improve benthic habitat quality are not necessarily in place (yet) in the entire area. Areas of concern are considered representative for the Dutch part of the North Sea in the sense that together they cover the most important habitats of the Dutch part of the North Sea. The areas of concern have functioned as search areas for specific management measures (especially closure for sea floor disturbing fisheries), although in practice nowadays measure have been taken in parts of those areas but also in areas extending beyond the initial borders of the areas. Nevertheless, the areas of concern are characterised by having their own representative benthic community monitoring, expected to be sufficient in effort for individual assessments.

[7] As a designated part of the 'closed area' related to the MPA Frian Front is a-typical in habitat constitution (situated south of the originally designated MPA; indicated as 'FF400', consisting predominantly of sandy habitat types instead of muddy habitat types), a BISI with a FF400-specific reference indicator species community is compiled.

The assessment tool was originally according to BISI v1; the current version according to v3 is a consolidation of original reference indicator species communities and based on boxcorer and benthic dredge dominated observation data, making use of the dedicated MSFD monitoring programme (Wijnhoven & Bos, 2017; Wijnhoven, 2022). With an exception for the HD area of the Cleaver Bank, HD habitat type H1170 and the BHT 'Offshore circalittoral coarse sediment' for which the BISI makes use of Hamon grab and video transect observations from the MSFD monitoring programme. Although the BISIs of BHTs could potentially form the basis of generic methodology for any other defined area or (HD) habitat (sub) type, this is currently not done for assessments at the level of the Dutch North Sea as the monitoring programme in place is according to a (consolidated) design developed to match the various HD – and MSFD areas for which assessment and reporting of quality status is needed under MSFD and HD.

- HD habitat (sub) types of the Dutch marine waters:
  The assessment tool includes BISIs for habitat (sub) types of the Dutch marine (and estuarine) 'Delta waters', the Wadden Sea and the coastal zone of the North Sea. BISIs are according to the protocol for generic application, compiled for HD areas, where relevant divided into habitat (sub) types by using (most distinguishing) ecotopes (with a standard BISI) as the basis for the derivation of indices following the ecotope surface distribution.
  BISIs are specifically compiled making use of ecotopes according to classification system ZES.1 (Bouma et al., 2005) for:
    - H1160 'Large shallow inlets and bays' in the Eastern Scheldt (Oosterschelde) based on 5 ecotopes distinguishing low - and high dynamic, littoral and sub littoral and low dynamic sub littoral further deviated into deep and shallow habitat.
    - H1130 'Estuaries' as present in the Western Scheldt (Westerschelde) and Wadden Sea (Ems-Dollard part) based on 8 ecotopes distinguishing meso - and poly haline, low - and high dynamic and littoral and sub littoral habitats.
    - H1110a 'Permanently flooded sandbanks of the tidal area' as present in the Wadden Sea (and smaller parts of the coastal zone of the North Sea, with regards to the BISI not distinguished from H1110b) based on 3 ecotopes distinguishing deep – and shallow habitat, the latest divided in low – and high dynamic habitat.
    - H1140a 'Intertidal mud flats and sandbanks of the tidal area' as present in the Wadden Sea (and three small scale sheltered HD areas adjacent to the North Sea coast, without specific monitoring) based on 3 ecotopes distinguishing high – and low

dynamic habitat, the latest divided in low – and middle to high lying habitat.
  - H1140b 'Intertidal mud flats and sandbanks of the coastal zone of the North Sea' as present in the North Sea Coastal Zone, Pro Delta, Western Scheldt and 2 smaller scale areas in the vicinity of the North Sea coastal zone, based on 2 ecotopes distinguishing sandy and coarse sediment habitat (of which the latest is only considered in case of extensive monitoring efforts as it covers only a small proportion of the total area under good quality conditions).

  The assessment tool has originally been developed according to BISI v2 (Wijnhoven & Van Avesaath, 2019) and is now in use according to v3. The assessments make use of the combination of monitoring under the MSFD programme, the Water Framework Directive (WFD) programme, Statutory Investigative Tasks with regards to shellfish surveys ('Wettelijke Onderzoekstaken; WOt) and Synoptic Intertidal Benthic Surveys (SIBES) programme extended to sub littoral sampling (under 'Waddenmozaïek') as well[8], sampling and monitoring using boxcorer/hand corer and benthic dredge/suction dredge/Van Veen grab/shovel'/oyster grab dependent of water bodies and specific environmental conditions in various combinations, however according to standardized methodology and designs (Wijnhoven & Van Avesaath, 2019).

- Generic assessment tool for OSPAR region II (Greater North Sea region):
  The Assessment tool is according to the generic methodology for assessment of random composite areas in the Greater North Sea region based on MSFD BHT surface distribution, distinguishing 6 BHTs as in use for the Dutch part of the North Sea as well: BHTs:
  - 'Offshore circalittoral coarse sediment' (including small part of mixed sediment habitat);
  - 'Offshore circalittoral sand';
  - 'Offshore circalittoral mud';
  - 'Circalittoral coarse sediment' (including small part of mixed sediment habitat; and in fact combining circalittoral and infralittoral coarse sediment habitat);
  - 'Circalittoral sand' (in fact combining circalittoral and infralittoral sandy habitat);

[8] Specific parts of WOt and SIBES monitoring (designated samples) are nowadays integrated as part of the official MSFD - and WFD monitoring programmes, providing amongst others information for HD (Article 17) reporting as well.

- 'Circalittoral mud'(in fact combining circalittoral and infralittoral muddy habitat);

The assessment tool has originally been developed according to BISI v2 (Wijnhoven, 2019) and is now adjusted according to v3. The assessments make use of standard core and grab samples of approximately 0,1 m² sieved over 1 mm mesh (common monitoring techniques in soft sediment habitats for most of the North Sea countries).

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 assessments, 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 assessments (e.g. with regards to MSFD, OSPAR, HD). Assessment of detailed habitats might for instance be useful in case of assessment 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 assessment of status of HD eco elements or OSPAR protected and declining habitats, like biogenic reefs and bivalve beds, that in the end can also be Other Habitat Types (OHTs) under the MSFD. 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 following ecotope surface distribution (see an example in Figure 6.3.2.a). Larger scale ecotope classifications for benthic habitats are generally/typically 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, 2021) 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 considered 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 assessment, however, might also need a comprehensive monitoring programme with sufficient representative monitoring for 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 considered as of significant importance to the overall quality status in

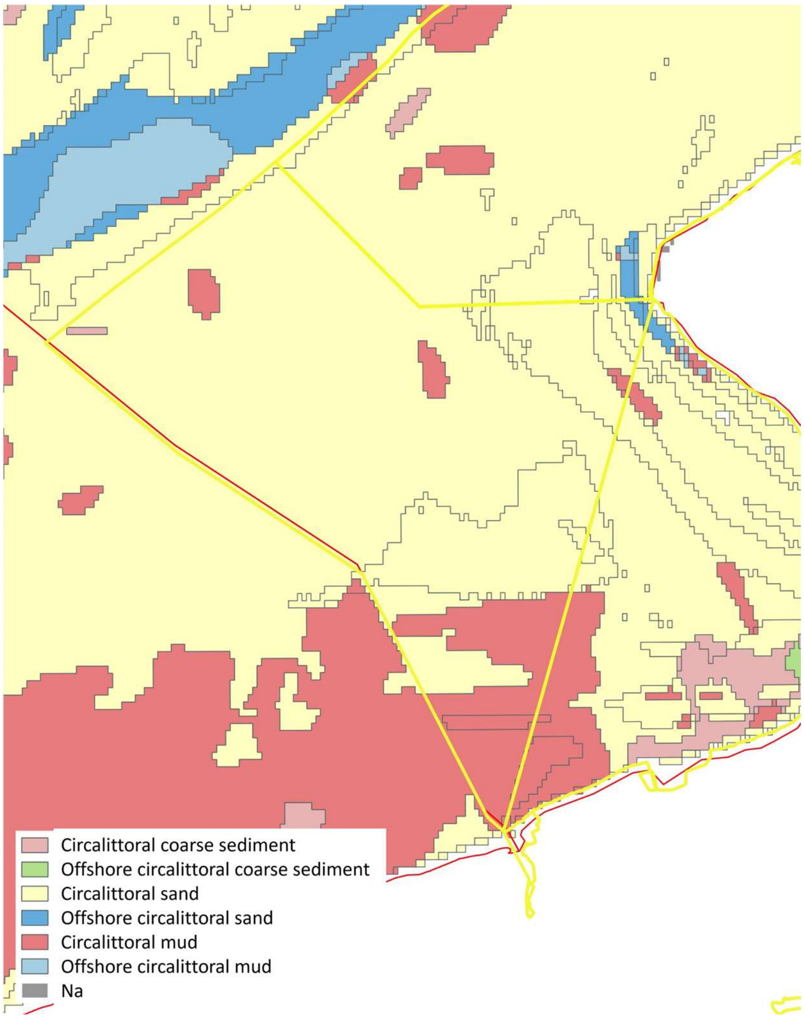


Figure 6.3.2.a. Example of Natura 2000 area 'Plain of the Raan' (area embordered by yellow lines in the middle) sub divided into ecotopes (BHTs) on basis of depth (offshore circalittoral, circalittoral) and sediment type (mud, sand, coarse sediment). Two ecotopes are distinguished on basis of which an area specific BISI is developed (circalittoral sand – 85%; circalittoral mud – 15%). In the Netherlands, the Plain of the Raan is designated HD habitat sub type 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 considered for the assessment of the 'Plain of the Raan'. A tiny part appears to be 'offshore circalittoral', but as this is a-typical 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 (characterized as 'offshore circalittoral') in the future (e.g. due to coastal works or sand extraction leading to 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 'circalittoral' now); combined as one BHT/ecotope in the current assessments 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.

terms of biodiversity or ecological functioning and can be included in that case).

In case of recurrent benthic habitat quality assessment (i.e. assessment of quality development for a fixed area over time), the habitat constitution might change over the years.

- **Real changes in habitat constitution:**
  In case of real changes in the habitat constitution, this is something one might be interested in.
  - Natural variability:
    Such changes might be, often small scale, natural developments in habitat constitution (e.g. due to succession and rejuvenation in communities or sedimentation and erosion processes). It is expected that such changes are more or less in balance on larger spatial scales or on larger time scales, and part of the variability in community composition, also under good quality conditions, and therefore taken care of in the area specific BISI and the reference indicator species community in particular. In practice a deviation from the good quality reference situation (i.e. BISI=1) is only considered as being of reduced quality in case of significant deviation (to take in consideration natural variability).
  - Anthropogenic impact and environmental change:
    Changes in habitat constitution might be the result of anthropogenic impacts, due to activities or structures, impacting or degrading the benthic habitats. Changes in habitat constitution, when expected to last more than 12 years, are considered habitat loss, at least when they occur at the level of BHTs, or when OHT disappear (Raicevich et al., 2024). In case of temporal changes this at least is expected to have impact on the benthic community composition and the indicator species community in particular. In case of working with a fixed habitat/ecotope map (not noticing on beforehand changes in habitat constitution) it is likely that changes are reflected in lower BISI scores (therefore the situation considered being of lower quality status), as in case the pressure does not directly impact the benthic communities it is at least expected that communities change towards species compositions in accordance with the new environmental conditions (habitat constitution). Such communities likely deviate more from the reference indicator species community of the original habitat/ecotope types. Moreover, such changes are often not gradual and additionally lead to pressure potentially leading to impact for sensitive indicator species (i.e. reduced abundances or even disappearance of indicator species). In case of environmental change (e.g. climate

related changes reflected in habitat constitution) the effect on benthic communities is about similar as in case of more direct anthropogenic impacts, although when not specific crucial threshold values are transgressed, changes (especially at community level) can be more gradual. However, alternative communities are expected in case of habitat changes, reflected in lower BISI scores in case of assessment using fixed habitat maps. Comparing the fixed habitat map with a more recent habitat map, and/or specific BISI assessments (e.g. focussing on quality developments of indicator species selection typical for certain conditions or particularly sensitive for changes of specific environmental conditions), might indicate the reason for changes in quality status.

- **Changes due to map updates:**
  Changes in quality due to map updates indicating (artificial) changes in habitat constitution.
    - Evolving and more accurate habitat mapping due to increased monitoring efforts or improved habitat models will impact quality assessment in case of the use of different maps for different assessments (in time). It is expected that before incorrectly indicated habitat type likely has been assessed as being of poorer quality, due to the presence of an a-typical indicator community, than when assessed according to the correct habitat type. In practice, benthic habitat quality status of composite areas is slightly underestimated to some extent due to incorrect habitat modelling. For comparability reasons it is recommended to consolidate the initial habitat map or, as the updated version is likely more accurate, use the updated map and redo former assessments according to the new map as well.
      In practice, map updates will often include a combination of improved habitat modelling and changes in habitat constitution due to anthropogenic impact and/or environmental change (and natural variability that is expected to be about similar for areas of concern through time), so that it is difficult to distinguish between the two.

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 'sea floor disturbance' and 'ecological disturbance (basically impact of nutrient enrichment 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:
    a. Potential size;
    b. Longevity;
    c. Frequency and number of recruits,

    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 0.75 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 ≥0.25) for the species, to be selected as an indicator species for the general quality status.
5) Species must have a possibility 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 assessed, references can be constructed from earlier references when identical or comparable ecotopes have been part of assessments before. However, improving data availability might influence reference

Table 6.3.3.a. Overview of the various BISI assessments and recommendation for the species-specific indicator values (iv_{is}). Besides the general quality assessment, these include specific assessments to identify potential causes for the observed quality status and potential effects of the observed quality status on ecosystem functions. Categories of specific assessments 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 0.75 for the combined categories C+D+E).

Code	**Causes and effects (to be assessed)**	**Description**	**Species specific indicator value (iv_i)**
General quality		Selected indicator species according to the five criteria as indicated in the text: Potential indicator species for relevant ecotope; either characteristic, indicative for a dominant disturbance or potentially 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, 0.125, 0.25, 0.375, 1)
B.	Ecological disturbance	Combined indicator value for effects of nutrient enrichment, pollutants and toxicants, and potentially hypoxia.	5 levels (0, 0.125, 0.25, 0.375, 1)
C.	Intensity of sea floor disturbing fisheries	Indicator value on basis of potential 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 (0.125, 0.25, 0.375, 1)
D.	Frequency of sea floor disturbing fisheries	Indicator value on basis of age of species (species that potentially can 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 20, 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, 0.05, 0.25, 1)
F.	Characteristic species	Species are almost exclusive or are much more abundant in the area of assessment than elsewhere (identification of being characteristic at ecotope level is a criterion for indicator species selection).	3 levels (0, 0.25, 1)
G.	Food web structure	Species important as food sources for higher trophic levels (i.e. fish, birds, marine mammals).	3 levels (0, 0.25, 1)
H.	Habitat diversity	Species creating permanent structures providing niches for a range of additional species.	4 levels (0, 0.125, 0.25, 1)
I.	Biological activation of sea floor top layer	Bioturbating and bio irrigating species with an important role towards ecological functioning (e.g. nutrient cycling, degradation of pollutants, providing suitable habitat for other species).	5 levels (0, 0.125, 0.25, 0.375, 1)
Specific assessments already in use in the Netherlands for specific areas, habitats or applications:			

Habitats Directive typical species; Indicators of increased hydrodynamics; Indicators of increased mud content; Indicators of increased inundation duration; Indicators of sea floor subsidence; Indicators of indirect effects of sediment extraction and – suppletion (increased turbidity water column). Other assessments that might be of interest: Indicators of impacts from exotic species; Indicators of temperature increase.

species lists and/or reference levels due to new insights. Occasional validation of reference values might be necessary during the years.

A BISI consists of an overall quality assessment (general quality index) for which indicator species selection is standardized as described before. A general quality assessment comes with a series of specific quality assessments (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 assessments 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 assessment (see example indicator species selection and valuation in Table 6.3.3.b). It is allowed to add additional species to the specific assessments (specific assessments are therefore less standardized and less comparable between different areas than the general BISI scores), to enlarge the number of indicator species in the specific assessment. 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' (for example 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 targets of assessments and in which time frame significant results can potentially be expected if present. Optionally sampling efforts, frequency and/or design can be adjusted accordantly.

Ideally, the power of specific assessments is calculated and presented with the results. The identified specific assessments as part of the BISI assessment tools, partly in use as criteria for indicator species selection for the general quality assessments 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 assessments (e.g. additional pressures of importance) arises. Specific assessments C, D, E and F have a role in indicator species selection for the general quality assessment and are therefore essential to be defined for BISIs in other regions as well. Specific assessments A and/or B can potentially be replaced by other (most) important pressure(s) for the area of concern.

The assessment 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

Table 6.3.3.b. Example of indicator species selection at the level of ecotopes (in this case according to ZES.1; Bouma et al., 2005) with indication of used sampling technique (assessment 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 >0.25 species indicative for specific ecotope) and indicator value (iv_i) towards different specific assessments (value between 0-1). All indicator species are included in the assessment 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) assessments 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 assessed by combining ecotope specific references following ecotope surface distribution (or assessments can take place at the level of individual ecotopes, and optionally BISI scores can be combined following ecotope surface distribution).

HD habitat type H1160	Ecotopes (ZES.1)							Pressure indicator								Recovery	Ecological functioning			
									Sand hunger											
	High-dynamic Sublitoral	Low-dynamic Sublitoral Deep	Low-dynamic Sublitoral Shallow	High-dynamic Litoral	Low-dynamic Litoral	Sampling technique	Indicator species general quality	Characteristic species	Increased hydrodynamics	Increasing inundation time	Ecological disturbance	Seafloor disturbance	Size potential	Longevity	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	0,125	0,125	1	(Box)core	1	1	1	1	0	0,25	0,375	0,3	1	1	0	0	1
Bathyporeia pilosa	0,125	0	0	0,125	1	(Box)core	1	0	0,25	1	1	0,25	0,125	0,05	1	0	1	0	0
Bathyporeia sarsi	0,125	0	0	0,125	1	(Box)core	1	0	0,25	1	1	0,25	0,125	0,05	1	0	1	0	0
Carcinus maenas	0,25	0,25	1	0,25	1	(Box)core	1	0,25	0,25	0	0,25	0	0,375	0,3	1	1	0,25	0	0,25
Cerastoderma edule	0	0	0,125	0,125	1	Dredge/grab	1	1	1	1	0,25	0,25	0,375	0,45	0	1	1	1	0,25
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.*

assessment as well. As the BISI methodology is in principle based on a static ecotope map, areas in change (often as a result of human related activities like large scale constructions; e.g. windfarms, artificial islands, sand extraction and suppletion, dikes, embankments and construction of extensions into the sea) or areas subject to large-scale developments (sea level rise or changed currents due to climate change or seabed lowering due to gas extraction), will likely result in observed benthic habitat quality differences as indicated by developments in BISI scores. When large changes in the seabed habitat constitution are expected; changes in BISI scores can be compared to ecotope difference maps (comparing ecotope surface distribution 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 (Wijnhoven& Bos, 2017). 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 assessments. So increasing the number of indicator species might to a certain extend lead to improved power when added species are really sensitive and specific; otherwise the power might decrease.

Samples should however also be representative for the area of concern (e.g. in spatial representation and covering environmental conditions and abiotic characteristics). 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 only an indication of the essential/least number of indicator species and samples included in an assessment can be provided. It is always necessary to test for the achieved power for the assessment, considering the actual quality status level, on a regular basis.

Testing (bootstrap methodology selecting sets of samples with varying sample size –with replacement– from relatively large data sets) provides some evidence on the relation of numbers of indicator species and the essential number of samples to obtain a certain power for assessment of quality status based on BISI. Figure 6.3.3 shows an example from the Eastern Scheldt (BISI for HD habitat type H1160), for which the available number of samples (38 boxcorer and 15 benthic dredge) available for 2012 are expected to provide a representative view of the general quality status. To test for possible impacts

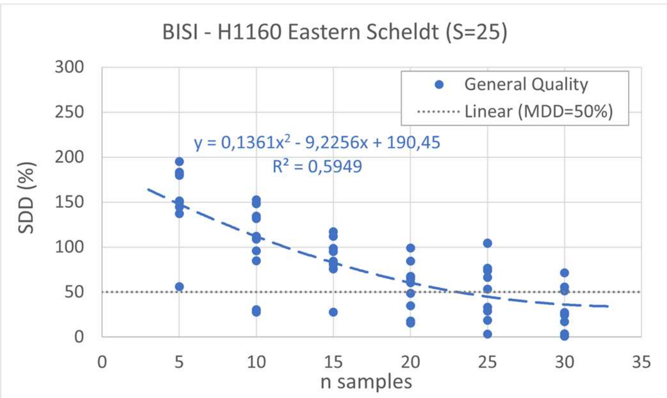
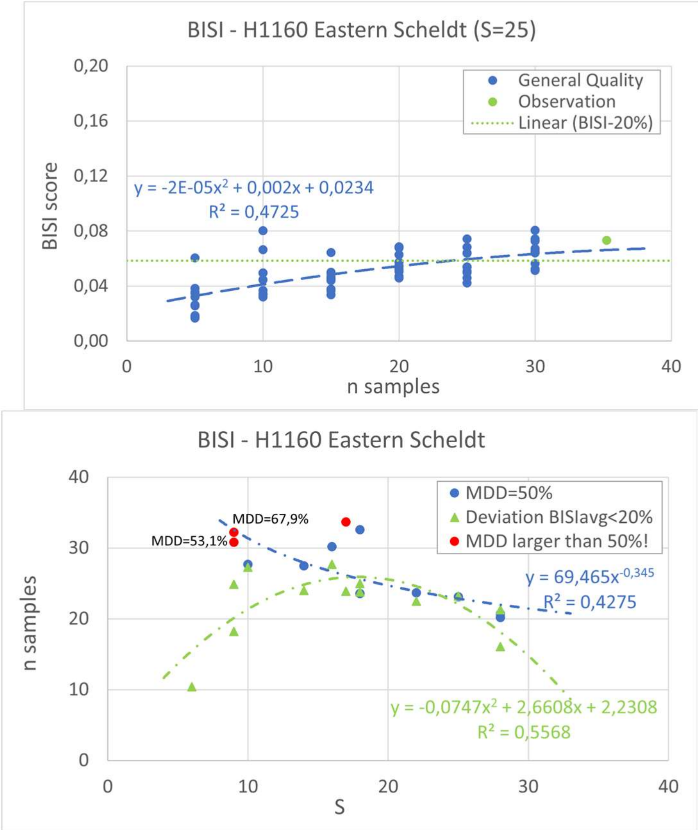


Figure 6.3.3. Example from HD habitat type H1160 in the Eastern Scheldt showing the relation between the number of indicator species included in the BISI (S) and the number of samples (n) needed to achieve a power of approximately 0.8 in



detecting 50% differences in the BISI score at $p<0.05$. a) Results of calculated significant detectable difference (SDD[1] in %) in general quality BISI (consisting of 25 indicator species) for random selections (with replacement) of a decreasing number of samples from the original data set of 38 boxcorer and 15 benthic dredge samples showing that on average a SDD of 50% is reached at 23,1 samples. b) Deviation of BISI scores from the observed BISI for the same random selections as for a. showing that on average the deviation in BISI score is trangressing the 20% at 23,2 samples. c) Overview of estimated number of samples needed to achieve an on average minimum detectable difference (MDD) of 50% and achieve on avarage a deviation in BISI score of less than 20% as obtained for specific BISI assessments (that consist of alternative numbers of indicator species as indicated) calculated for the same random selections as for a. and b.[2]. In three cases a MDD of 50% cannot be achieved (alternative minimum MDD value that can be achieved is indicated).

[1] *Significant Detectable Difference (SDD) calculated as the absolute values of the critocal t-value divided by the computed t-statistic times the (observed) BISI score divided by the difference between observed and estimated BISI score times 100%* $(=ABS(t_{critical}/t_{computed}/(100*(BISI_{observed}-BISI_{testset})/BISI_{observed})))$.

[2] *Indicated trends in c., only indicative showing increase in variability with increasing and decreasing indicator species numbers.*

of using alternative numbers of indicator species, the results of specific assessments are used (see 'BISI v3 Assessment tool for marine HD habitats' available from 'http://ecoauthor.net/bisi/' for details about specific assessments). It has to be noticed that results are always to some extent determined by relative indicative value of the set of indicator species for a certain aspect of quality, and the extent to which other aspects (natural variability and other pressures) lead to variability and/or impact specific BISI scores. To achieve a certain power of assessment (in this case at least differences of 50% in BISI score can be significantly detected at $p<0.05$ -one-sided independent t-test-, and on average assessed BISI scores should not deviate more than 20% from the observed BISI score, to reflect a power of 0.8), in case of the Eastern Scheldt with application of the combined sampling technique BISI, at least about 23,2 samples are needed for general quality status assessment (Figure 6.3.3.a,b). This is the essential weighted average number of samples for the two monitoring techniques (present monitoring -the observation- consists of a weighted average of 35,5 samples) to achieve the indicated power of general quality status assessment.

From power analyses for specific assessments it can be learned that in case the included indicator species number is around 20 to 25, the essential (weighted) number of samples is about 25 to allow both detection of at least 50% differences and on average less than 20% deviation from the actual BISI score (Figure 6.3.3.c). In case of lower indicator species numbers, the essential number of samples to achieve the indicated power starts to deviate. On one hand, the impact of natural fluctuations in populations and/or possible impacts from other disturbances or environmental changes leads to increased variability at lower indicator species numbers. On the other hand, certain quality or ecological functioning aspects might be best described with a limited number of the most sensitive/indicative species. It has to be noticed that for several specific assessments the proposed power appears to be out of reach (MDD larger than 50%), suggesting that either more indicator species should be included, that selected indicator species are not sufficiently specific, or that the type of disturbance or quality aspect is not among the most important for the area of concern or not well defined. Higher variability in essential sample number with higher indicator species numbers might also indicate that less indicative species are included in the assessment. In the current example the essential number of samples to achieve the proposed power is however expected to be lower (but not much lower) than the indicated 25, so that in this example it can be considered an indicative value at the safe side in case the BISI consists of more than 25 indicator species. Be aware that natural variability in habitat constitution or in environmental conditions in the assessment area of concern, or the use of alternative observation techniques, might lead to alternative essential numbers of indicator species and samples. It is therefore recommended to do power assessments in case of new types of applications and to repeat power assessments when substantially more (benthic community observation) data have come available.

6.3.4 Assessment relative to compiled reference levels

There are pros and cons of using reference levels in assessments. 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. A methodology is needed which besides on the long term is also potentially capable of showing improvements on the short and mid-long term, so that effects of management regulations and measures can be detected within management cycles. Therefore there is not much use of comparison with pristine conditions, as unrealistic reference levels would only mask changes (if there are) as relative differences between reference levels and observations are expected to be small.

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 either the abundance of the indicator species is still at a poor quality level or that a level is reached where no further quality improvements are expected/necessary (or an alternative quality state in between).

For each area to be assessed, an area specific (internal) reference is defined/constructed. Where initially (BISI v1) references were defined at the level of individual assessment areas, at present (with BISI v3 and v2) internal references are specific for ecotopes and can potentially be used for different areas within the same sub region where the same ecotope is present. Exceptions are potential indicator species that in certain regions have no opportunities of colonizing the assessment area of concern in a natural way. Such potential indicator species should be excluded from the reference or be replaced by region specific indicator species. For assessments of other types of areas and habitats (compiled areas), internal references are composed from the references of the (most important) ecotopes, following surface distribution.

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 assessment) compare to recent historic observations. As complete standardisation seems to be impossible, and some room for interpretation and application of best insights is expected to be beneficiary, this scheme is mainly intended to provide (some) guidance in derivation of realistic references. It is recommended to use references at the level of individual ecotopes as the basis and re-use those references as much as possible for compiled assessment areas. It has to be noticed that a lot of effort can be put in the derivation of reference levels for indicator species, whereas the absolute value might not be the most important aspect due to logarithmic scales and the fact that also increase in occurrence to (100 times) above reference level lead to increase of the BISI score. It is more important whether species are present or not and whether

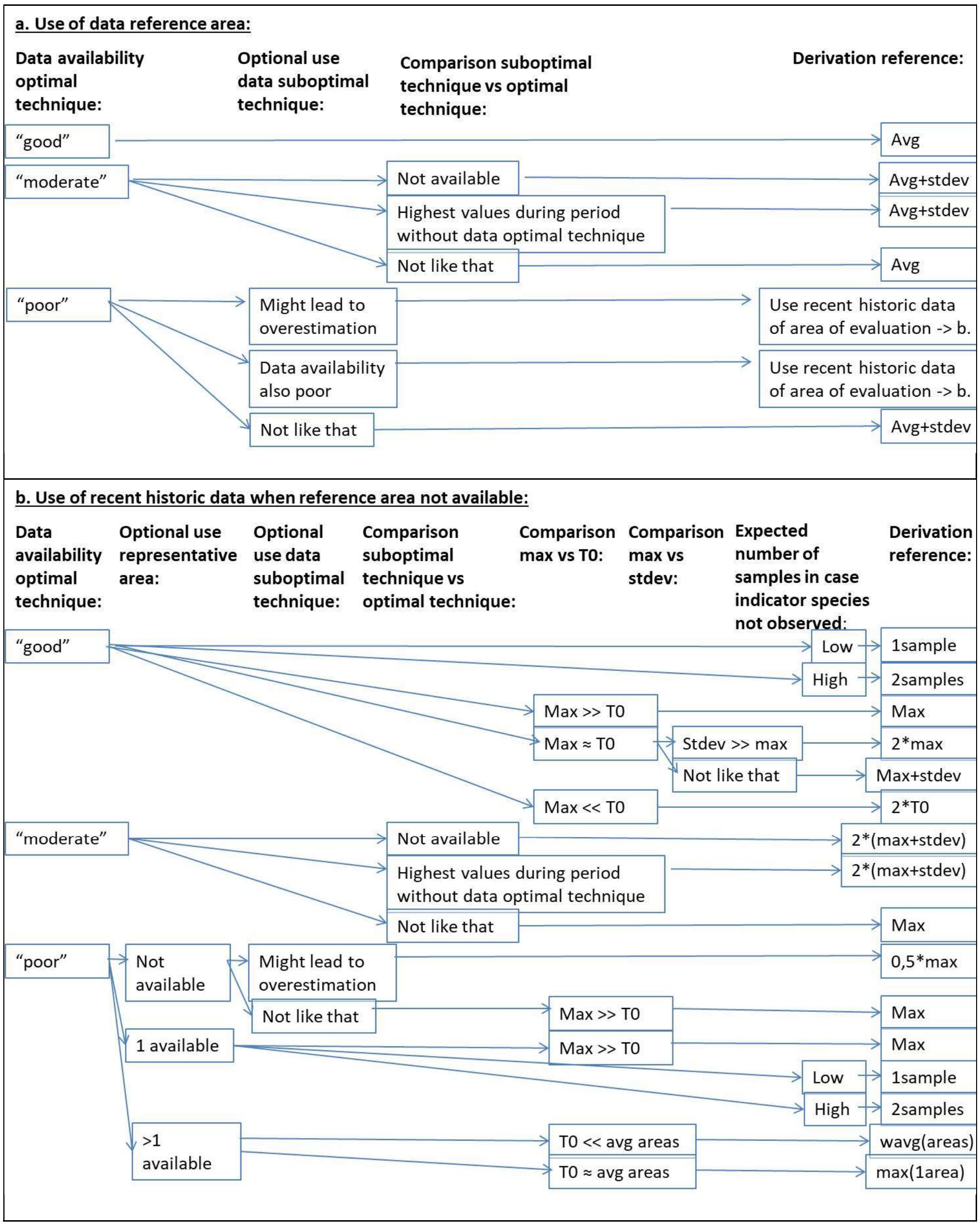


Figure 6.3.4.a. Flow chart showing the decision schemes of how to derive area/ecotope specific reference values for individual indicator species. a) Scheme to use in case a reference area is available; reference values can potentially be obtained from monitoring data of a reference area (e.g. results from typical species composition identification in uni-pressure - to reference area comparisons or extracted from gradient studies). 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 assessment methodology is developed and to which future assessments will be compared; 1sample = a density similar to an occurrence (1 specimen) in one sample; wavg = weighted average of several areas based on the number of samples taken per area; >> = much larger; << = much smaller; ≈ = comparable values.

there are changes in relative occurrences (see Figure 6.4.2 for the conceptual behaviour of the BISI as well).

As the derivation of reference values and the construction of an internal reference for areas to be assessed are based on ecotopes, in practice data from corresponding ecotopes are distinguished for reference areas, after which a suitable reference might be constructed following ecotope surface distribution. 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 (in OSPAR or with regards to the MSFD generally a sub region). Potential indicator species that cannot return to the assessment area 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 as observed from recent historic monitoring data of the area of assessment itself, that are adapted, doubled and/or increased with the standard deviation. Derivation methodology depends on the (recent) 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 assessment (as densities were too low) during recent years.

Table 6.3.4.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 ZES.1 as indicated in Table 6.3.3.b) and calculated into reference occurrences (R_i) for the Eastern Scheldt, following ecotope surface distribution (as indicated by the formula). 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 assessment/comparison (e.g. specific technique in specific habitat or an 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.*

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). 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 6.3.4.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 following ecotope surface distribution 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 6.3.4.c 'large shallow inlets and bays'; H1160) in the same geographical (sub) region. Monitoring programmes should match the purpose of the assessments (give a representative view of the quality status of areas or aspects of concern); and selection of the suitable monitoring data might be necessary. In case monitoring programmes are not representative for entire regions (e.g. sample numbers not reflecting ecotope/habitat surface distribution or samples with a skewed spatial distribution), assessment 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[9] 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 assessment (where it is indicated when especially in the historic data or in assessment 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 considered 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.

[9]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

Occurrences (O_i) of indicator species for the assessment year(s) and area(s) of concern are calculated from observation data of appropriate (observation) methodology, data type and sample type as indicated. The observed variance in the observation data (occurrence) is included in the assessments. 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. observed occurrence 100x larger or smaller than the reference occurrence). 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 truncation in case O_i deviating more than 100 times from the reference occurrence; R_i) of occurrence to reference ratio (O_i/R_i) with standard deviation (stdev; adjusted to 0.01 in case occurrence without variance). 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 (occurrence data that can be replaced by observations in case of BISI assessment for alternative situations/years).

HD habitat type H1160		Eastern Scheldt				
Indicator species	Sampling technique	R_i	O_i (2012)	±stdev	O_i/R_i (2012)	±stdev
Arenicola sp.	Boxcore	53,093	8,381	36,787	0,158	0,693
Bathyporeia pilosa	Boxcore	86,865	0,000	0,000	0,010	0,010
Bathyporeia sarsi	Boxcore	41,142	3,352	14,414	0,081	0,350
Carcinus maenas	Boxcore	21,837	1,676	10,333	0,077	0,473
Cerastoderma edule	Dredge/grab	106,807	7,333	16,676	0,069	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[10]' with regards to general quality assessments), specific assessments are performed on weighted species subsets. For specific assessments, indicator species lists can optionally be supplemented with additional species (depending on the specific indicator value of species for certain causes of change in quality or potential effects of change in quality status). Depending on the type of assessment (general or specific), O_i/R_i ratios are multiplied with the species and assessment specific weights (indicator values; IV_i's calculated as the species specific indicator weight iv_i as shown in Table 6.3.3.b, divided by the average

[10] 'Smart species' are potential indicator species that are either sensitive, typical, characteristic or have specific traits of relevance towards ecological functioning aspects, for which potential differences in occurrences can be observed with realistic monitoring efforts and techniques (resulting in sufficient power in statistical testing/assessments) as described in Wijnhoven et al. (2013).

indicator weight iv_{avg} of all indicator species included in the assessment, to achieve comparability in assessment results for all assessments as indicated) resulting in values as shown in the example of Table 6.3.5.b. To calculate the geometric mean of sets of indicator species results, the natural logarithm of the ratio, multiplied by the indicator value, is taken; here indicated as the Individual Indicator Species (IIS_i) result. The use of a logarithmic scale 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. To calculate the BISI score (assessment result), the IIS_i values are summed, divided by the assessment specific number of included indicator species, and back-transformed taking the exponential (inverse natural logarithm). Similarly, the corresponding standard deviation is calculated by summing the calculated variances of the IIS_i values ($Variance_{IIS} = Stdev^2/(O_i/R_i)^2$), divide those by quadrated BISI scores, and take the square-root of the result.

To summarize the resulting BISI equation equals:

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

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 realistic reference (BISI=1) value. Working with the BISI making use of 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 assessments

Table 6.3.5.b. Example of calculation of the BISI including standard deviation. O_i/R_i ratios at the level of individual indicator (as calculated before: E.g. Table 6.3.5.a) species of which the natural logarithm is taken, are multiplied with the species and assessment specific indicator value ($IV_i = iv_i/iv_{avg}$). The results are summed and divided by the number of indicator species in the assessment, after which the inverse natural logarithm (e to the power of the product) of the result 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 taking the squared 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. 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	**IISi=IVi*ln(Oi/Ri)**												
		Importance at national level	Pressure indicator						Recovery indicator	Importance at national level	Ecological functioning indicator		
Indicator species	General quality	Characteristic species F	Increased hydrodynamics K	Increasing inundation time M	Ecological disturbance B	Seafloor disturbance A	Size potential C	Longevity D	Frequent recruits potential E	HD typical species J	Foodweb importance G	Habitat diversity importance H	Biological activation importance I
Arenicola sp.	-1,846	-2,461	-3,304	-2,014	na	-1,231	-2,628	-1,484	-1,911	-1,846	na	na	-3,527
Bathyporeia pilosa	-4,605	na	-2,060	-5,024	-8,059	-3,070	-2,186	-0,617	-4,768	na	-5,526	na	na
Bathyporeia sarsi	-2,507	na	-1,122	-2,735	-4,388	-1,672	-1,190	-0,336	-2,596	na	-3,009	na	na
Carcinus maenas	-2,567	-0,856	-1,148	na	-1,123	na	-3,655	-2,064	-2,658	-2,567	-0,770	na	-1,226
Cerastoderma edule	-2,679	-3,571	-4,793	-2,922	-1,172	-1,786	-3,814	-3,230	na	-2,679	-3,214	-3,061	-1,279
BISI = exp((1/S)*∑(IV$_i$*ln(O$_i$/R$_i$)))	**0,073**	**0,054**	**0,079**	**0,093**	**0,051**	**0,063**	**0,056**	**0,041**	**0,089**	**0,086**	**0,063**	**0,062**	**0,134**
± stdev	1,153	0,519	1,460	0,978	0,661	1,047	0,967	0,678	1,375	0,926	0,913	0,348	2,111

(e.g. with regards to the MSFD) could be a BISI value of at least 0.5 for a sufficient good quality status in 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 needs quality improvement (in line with amongst others MSFD and HD assessment cycles).

Initially the BISI methodology was developed to compare the quality status of sea areas to a certain initial situation (T0). Specific assessments 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.

Working with compiled BISIs following ecotope surface distribution (using the same references for the same ecotope in different areas), the design of the area specific index is more standardized. Herewith the comparability of quality assessments between different areas has been improved. Application in various circumstances and different areas, including possible reference areas, can support fine-tuning of the current methodology. It should be considered 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 Quality status assessment and significance testing

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

Initially the assessment methodology was specifically developed at the level of management areas like in case of the Dutch part of the North Sea, assessment of Marine Protected Areas (MPAs including Natura 2000 areas and MSFD areas), Habitats Directive habitat types (that include (parts of) MPAs but generally extent outside MPAs), and MSFD Broad Habitat Types (BHTs, basically similar to EUNIS level 3 ecotopes, that cover areas partly inside and outside MPAs). With the current BISI v3, indicated assessment areas can still be core targets for assessment. However, ecotopes or MSFD broad habitat types (proposed EUNIS level 3; EUSeaMap, 2021) or comparable ecotopes like aggregations of those or classifications according to other ecotope classifications like ZES.1 (Bouma et al., 2005) form the basis to construct area specific assessment indices following the ecotope surface distribution in the assessment area. 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 assessment of the quality status of specific areas, the assessment methodology for the Dutch North Sea region was initially developed including special assessments 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 over the distinguished ecotopes.

For each of the indicated areas to be assessed 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 broad-scale 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. Starting from BISI v2 the scale is adjusted (normalized) so that a BISI score of around 1 is considered a realistic good quality (reference) 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 BISI v3 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 (e.g. Raicevich et al., 2024).

Basically, all kind of testing can be applied to the 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 assessment 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 (see example in Table 6.3.6.a). Additional 2-sided independent t-testing of the quality status of different areas (Table 6.3.6.a) or the same area in time; that can be a 2-sided paired t-test (e.g. compared with a T0 situation before management regulations 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 developments 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.

HD habitat type H1160		Sand hunger	
		Increased hydrodynamics	Increasing inundation time
	General quality	K	M
BISI	**0,073**	**0,079**	**0,093**
±stdev	1,153	1,460	0,978
1-sided independent t-test (with reference)			
Pooled Std Dev	1,153	1,460	0,978
Computed t Statistic	16,870	10,711	11,273
Critical Value of t	1,961	1,962	1,964
Probability of Computed t	0,000	0,000	0,000
Significance	***	***	***
1-sided independent t-test (among specific assessments)			
Pooled Std Dev		1,139	
Computed t Statistic		0,167	
Critical Value of t		1,963	
Probability of Computed t		0,434	
Significance		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 assessment and both specific assessments). 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 to sample number ratio. The critical t-value equals the inverse of the one-tailed Student's t distribution calculated in Excel with the T.INV function considering 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 considering a t-distribution (T.VERD in Excel) over the absolute value of the computed t statistic, considering the degrees of freedom.

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 assessment potential initial differences in benthic indicator species assemblages (at T0) are considered. 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 assessment. 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 assessment using a one-sided t-test.

6.4 Assessment benchmark and conceptual behaviour

6.4.1 Detecting good or adversely affected quality status

Dependent of goals, targets and context there might be different interpretation of what to consider good quality status.

Conservation and improvement objectives are common targets of marine policy and management and typical goals for areas like MPAs or specific habitats, like for instance common practice in Natura 2000 policy (including the HD), quality assessment and evaluation. In that case one or several initial assessment(s) provide the starting or T0 situation and quality status of often before specific measures have been taken. Such a T0 situation for comparison can also be a specific year or period in the past (although data availability and representativity in case of absence of dedicated monitoring programmes in the past might make it difficult to statistically demonstrate quality consolidation or improvement). In case of (sufficient) data availability there are potentials to test for the absence of decreasing trends or particular testing for presence of increasing trends, alternative the testing for presence or absence of year-to-year differences of assessment years compared to T0 situations. It is always important to show the power of testing (with regards to the realised design) as the power of the test/monitoring design could determine whether it is likely that targets are met. Nevertheless, when the design is considered acceptable, the combination of BISI scores, (natural) variability in the form of (pooled) standard deviations, and cases (number of samples either or not combined with indicator species numbers) allows straight-forward significance testing if the form of singular of multiple t-testing at defined significance levels.

However, good quality status is not just a random level and therefore also not a random BISI value. Benthic habitat good quality status is the least status at which abiotic and biotic characteristics (e.g. typical species presence and benthic community composition, presence of sensitive species and species with key functions, functioning of ecosystem processes) reflect the prevailing natural physiographic, geographic, climatic/environmental and biological conditions but may show an acceptable deviation from reference state (Raicevich et al., 2024). Quantification of what exactly is an acceptable deviation, and what is a community including sufficiently above mentioned aspects, is matter of international discussion and alignment, currently taking place amongst others within and in a process under the guidance of the EU Marine Strategy Framework Directive Common Implementation Strategy Technical Group Seabed (TG-Seabed) with regards to MSFD assessments. It is however also a topic amongst others discussed at Regional Sea Conventions (e.g. OSPAR, HELCOM; actually also involved in the above mentioned MSFD-related process). Above mentioned process of quantifying good benthic habitat quality status should lead to threshold values (in this case with regards to MSFD descriptor 6 criterium 5; 'the extent of adverse effects from anthropogenic pressures on the condition of habitat types') for MSFD BHTs and OHTs and a tuning of potential suitable benthic habitat quality indicators to a common scale. It has to be noticed that benthic habitat quality besides pure quality aspects also includes extent aspects. It is therefore not necessary that all benthic community aspects are present on each sample side, but it could be in a certain extent of area, moreover most ecological functioning aspects do need a certain surface area and possibly the combined presence of various habitat types and/or low level of fragmentation. At present it is foreseen that the process of defining commonly accepted threshold values describing good benthic habitat quality condition will run at least until the end of 2025. Nevertheless the qualification of good environmental status (GES) so far (Raicevich et al., 2024) provides handles to adjust the standardized quality status assessment results (in the form of BISI scores) too, as the conceptual behaviour of the BISI is known (see chapter 6.4.2).

Additionally, although realised monitoring efforts should be sufficiently large and not play a role in what is considered a good quality status, in practice indicating a certain BISI score as the threshold value for good quality status, whereas the value does not meet the detection limits in terms of power of design to identify possible significant differences, is not of much use. At the moment, the power of the designs of the monitoring programmes in the Netherlands around areas and habitats in the Dutch part of the North Sea and for marine HD habitat types, take into consideration that at least differences of 50% in BISI score between two observation moments should be detectable. This means that a BISI<0.5 for an assessment areas can be distinguished significantly from the internal reference value of BISI=1. This suggests that based on current designs, a threshold value for benthic habitat quality should not be larger than 0.5, as in that case situations can occur where benthic habitat suffering adverse effects cannot be distinguished from good quality reference situations (unless monitoring is intensified).

6.4.2 Conceptual behaviour of the BISI

Assessment on basis of BISI leads to a benthic habitat quality scores (BISI values) 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.

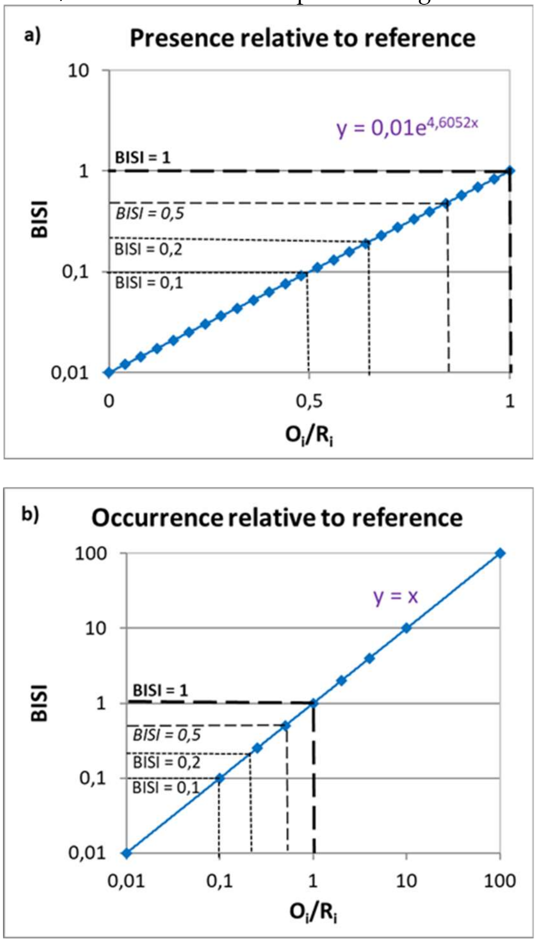


Figure 6.4.2. Response curves of BISI in case all indicator species have the same 'weight' in the assessment (Indicator value IV_i of all species = 1). a. Response of BISI score when presence/absence of indicator species changes. The x-axe indicates the share of indicator species present relative to the number of species forming the internal reference. (An occurrence at reference level is assumed for indicator species 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 = $\exp((1/S)^* \Sigma (IVi^*\ln(Oi/Ri)))$, with IV_i=1 in this example (all indicator species have similar weight) and approaches BISI = $0.01^*\exp(4.6052^*(O_i/R_i))$. 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. BISI=1 equals the reference situation and examples of other BISI scores (0.5, 0.2 and 0.1) are indicated.

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. 6.4.2.a) from the effect of changes in occurrences of species (Fig. 6.4.2.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 (IV_is) of individual species result in a slightly larger impact of differences in occurrence of the one species (larger IV_i) on the resulting BISI score than from the other (lower IV_i).

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. 6.4.2.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 many indicator species are missing. The impact of abundance of indicator species on the observed quality status (BISI score) is a linear response (Fig. 6.4.2.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.

From Figure 6.4.2 it can be learned that at BISI=0.5, this means that either around 15% of the indicator species is absent, or that indicator species occurrence is 50% of the reference occurrences; in practice it is likely that a combination of the two, with in that case lower reduction values for both. This might for the time being, be an acceptable threshold value for the BISI that seems to be in line with the qualitative description, until the international derivation process (see chapter 6.4.1) has led to broadly agreed proposals to which BISI values can be adjusted.

6.5 Data source and description of data

There are two types of essential benthic data that are needed for application of the BISI in new areas. 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 BISIs, but are required for the selection of indicator species and estimation of the reference values. However, in case of already defined indicator species lists and reference occurrences at the level of ecotopes or habitats from the (sub) region of concern, by which the assessment area can be described, such historic data are not necessary (and possibly only of use for validation of already defined indicator species reference communities).

Then there are the benthic community observation data, that are preferably representative for the assessment area, or at least parts (e.g. specific ecotopes/habitats) within the assessment area, on which the assessment takes place. These data likely include data of some kind of initial state (T0): A situation that is assessed or where other assessment moments (possibly in the future) are compared with. Characteristics and essentials of both data sets are described below.

6.5.1 Data to extract reference levels

Although 'historic' might suggest that observations from decades to centuries ago are necessary; 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. 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 besides quality improvements (or deterioration) on the longer term, also changes on the short - and mid-term, potentially as a result of changes in management and taken measures. Therefore a more realistic reference has to be used, based on current habitat constitution and present species pools, potentially showing first indications of quality improvements and/or deterioration. 'Compiled reference levels' should reflect a realistic target in case the dominant pressures are reduced. In (sub) regions with few disturbances and plenty of reference areas, the realistic reference might approach pristine reference situations, but generally (like for the Southern North Sea region and the Dutch part in particular, for which the BISI has initially been developed) realistic reference communities are not comparable to pristine reference situations.

The derivation of internal reference levels, although monitoring efforts, representativity 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.

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.

It might be the case that on the long term it might be necessary or beneficiary to adjust used references. It could be that additional monitoring and/or successful conservation or restoration measures provide new insights in what the realistic reference communities could look like. It should be mentioned that references can easily be adjusted, however that it asks for redoing former assessments (using the adjusted reference) of the same kind (same area at other moment or areas to compare with) as well. The derivation of the internal reference is standardized (as much as possible) according to the scheme presented in Figure 6.3.4.a, and includes besides derivation on bases of observations from reference areas or situation, the derivation of the realistic reference on basis of maximum potential occurrences as extracted from recent observation data. Besides maximum occurrences, also standard deviations and patterns in occurrences in other data sets (neighbouring areas or sub optimal 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 assessments

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. boxcorers and benthic 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 assessment, 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 time frame) and a significance level is considered. It is expected that essential number of samples in generally within reach, as power analyses are part of indicator species selections as well, so that only 'smart' indicator species are part of the general quality assessment BISIs. If certain specific assessments are of importance, it might be that the number of necessary samples is higher in that case (or a lower power for these tests should be accepted). There might be options to spread out monitoring over several campaigns or years and combine data for specific assessments to increase 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 Interplay with other indicators and assessments

It has been acknowledged that even in case of an indicator with the potential to distinguish in relative importance of sources of pressure leading to impact reflected in the quality status and the potential to indicate effects from observed quality status on ecological functioning aspects (like the BISI), such an indicator should preferably be applied in combination with other indicators and assessments to come to a founded quality status assessment (Van Denderen et al., 2024). Various indicators focus on various quality aspects, and it is therefore suggested to combine benthic community observation based indicators focussing on typical and sensitive species (like the BISI) with total community or diversity related indicators. It is also valuable to assess quality status based on more than one type of observations (e.g. different observation techniques, monitoring designs, timing of sampling, etcetera; can optionally be combined in the BISI, but assessment with different indicators could be valuable as well) as it might focus on different aspects of quality. Besides, observation based monitoring often deals with a certain lack of data as programmes might focus on specific areas or sample density is lower than preferred. Therefore combining observation based assessments with pressure based impact modelling is almost a necessity to retrieve high spatial coverage of the assessment. It is therefore however helpful that the BISI can provide evidence on what pressures to focus on, as such information is generally lacking (or models focus on the presumed most important pressure, often just one pressure type considered). Observation based indicators like the BISI could shed a light on whether benthic habitat quality patterns are indeed explained by the presumed pressure type, or whether combined mutually affecting impacts possibly play a role.

The problem of relative 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 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 those limited data available.

7 References background information on methodology

The first steps leading to a Benthic Indicator Species Index (BISI v1) are 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.

The report focusses on the development of assessment indices for fixed areas which include MPAs, BHTs and HD habitat sub types of the Dutch part of the North Sea, and includes the area/habitat specific selection of ‘smart’ indicator species, combining occurrence data of different types from different observation techniques in one index/assessment, derivation of indicator species reference occurrences, presenting the combination of a general and several specific assessments including the first (ready to use) assessment tool[11] and the first version of this BISI protocol[12].

Since the first version of the BISI, the methodology including the formula has been adjusted and been presented as the BISI v2. The most important difference compared to v1 is that BISI v2 is geared for generic application in a variety of systems and the international context as well. With BISI v2, indicator species reference communities are defined at the level of ecotopes (or broad habitat types) from which (compiled) area specific BISIs can be composed following ecotope surface distribution. Additionally the selection of indicator species is better standardized including that reference areas have a more prominent role now in the derivation of reference occurrences, the formula has been adjusted with regards to the use of the natural logarithm instead of ^{10}log and there are adjustments to the calculation of the pooled standard deviations.

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[11] Wijnhoven, S. (2017). Assessment tool ‘Benthic Indicator Species Index (BISI)’; Application of BISI v1 in the Dutch North Sea areas of evaluation. v260917 (also Appendix 2 of Wijnhoven & Bos, 2017).
[12] Wijnhoven, S. (2017). Protocol Benthic Indicator Species Index (BISI)., v260917 (BISI v1). (also Annex 1 of Wijnhoven & Bos, 2017).

With the current version (v3) the sensitivity of the index to detect possible differences in quality status and to identify possible causes and consequences of benthic habitat quality differences in particular, are improved, by adjustment of the formula (placing the indicator value as a relative weight of species in the calculation, outside the log-term), and reducing indicator values in case species are not the most indicative (IV_i<1) species. Additionally the calculation of pooled standard deviations has been corrected accordingly.

Herewith although results from each of the BISI versions can be used separately and quality developments for defined areas will point in the same direction for general quality assessments with each of the versions, the absolute assessment results are not comparable and cannot be combined. It is suggested to use BISI v3 in case of new applications, and in case of recurrent assessments redo former assessments (based on earlier version) with BISI v3.

Besides this protocol, a couple of (ready to use) **assessment tools** (according to BISI v3) have been developed that include the application for MPAs, broad habitat types and HD habitat (sub) types of the Dutch part of the North Sea (with consolidation of earlier identified indicator species reference communities):

- Wijnhoven (2023a). Assessment tool 'Benthic Indicator Species Index (BISI)': Application of BISI v3 in the Dutch North Sea with consolidation of earlier identified references. v021023.

the generic application for Habitats Directive areas and marine HD habitat (sub) types in the Netherlands, based on standard monitoring programmes making use of a variety of observation techniques with however recurrent application:

- Wijnhoven (2023b). Assessment tool 'Benthic Indicator Species Index (BISI)': Application of BISI v3 for marine Habitats Directive habitat types of the Dutch 'Delta-waters', the Wadden Sea and the coastal zone of the North Sea. v061023.

the generic application in the Greater North Sea region using standard grab and core benthic community observation data and the use of defined indicator species reference communities for a selection of Broad Habitat Types:

- Wijnhoven (2023c). Assessment tool 'Benthic Indicator Species Index (BISI)': Application of BISI v3 in soft sediment habitats of OSPAR region II (Greater North Sea region). v031023.

A **recent application** is the benthic habitat quality status assessment as part of the Dutch Marine Strategy (MS1 part 1 – EU MSFD Article 8 reporting 2024; Min IenW et al., 2024) that will be published in 2024, for which with regards to D6C5 the background reporting of the BISI application (according to v3) is available as:

- Wijnhoven, S. (2023d). Beoordeling kwaliteitstoestand Nederlandse deel Noordzee op basis van de Benthische Indicator Soorten Index (BISI). Toestand en ontwikkelingen van benthische habitats en HR-/KRM-gebieden gedurende 2016-2021 in vergelijking tot voorgaande jaren. Ecoauthor Report Series 2023 – 02, Heinkenszand, the Netherlands. (in Dutch).

Other reporting around BISI applications (according to v2) are:

- Wijnhoven, S. (2021). Korte termijn effecten pilotsuppletie Amelander Zeegat. Analyse ontwikkeling benthische habitats met behulp van de BISI. Ecoauthor Report Series 2021 - 01, Heinkenszand, the Netherlands. (in Dutch).
- And the benthic habitat quality status assessment of specifically HD habitat (sub) types H1170, H1110b and H1110c as part of the HD Article 17 reporting included in:
  Janssen, J.A.M., Bijlsma, R.J. (eds.), Arts, G.H.P., Baptist, M.J., Hennekens, S.M., de Knegt, B., van der Meij, T., Schaminée, J.H.J., van Strien, A.J., Wijnhoven, S., Ysebaert, T.J.W. (2020). Habitatrichtlijnrapportage 2019: Annex D Habitattypen. Achtergronddocument. Wettelijke Onderzoekstaken Natuur & Milieu, WOt technical report 171. 97 pp. (in Dutch).
- 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 development of the BISI (v2) with regards to marine HD habitat types, is specifically described 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).

All products (reports, protocols -including former versions-, application tools and documents with regards to assessments) are available via the Ecoauthor website: www.ecoauthor.net, where a specific page on BISI (http://ecoauthor.net/bisi/) is present.

8 Strengths and weaknesses

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 assessments 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.

In case of focus of the BIS application (including a dedicated monitoring programme) on specific management measures, this provides 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 recent 'historic' (typically of the last three decades) observations, a realistic reference is ensured, that is within reach with effective management. Although the methodology is specifically developed to evaluate change in quality status in time or between differently treated areas (making use of a fixed monitoring design), a comparison of the 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 has been found that even an assessment based on one sampling methodologies (i.e. which generally also means just half of the indicator species involved, like in case of the Dutch North Sea areas quality status assessment solely based on boxcorer samples or only based on benthic dredge samples instead of the combination of the two) 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.

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 habitat surface distribution. It is likely that the loss of highly valuable ecotope (expected to be species rich or diverse or important for unique species not common in other ecotopes or with important ecosystem functions) will also lead to a lower BISI score and *vice versa*.

Benthic habitat quality assessment takes place at the level of areas/habitats/ecotopes with sufficient and representative monitoring; i.e. based on several samples/observations. Herewith also naturally less common species, typically occurring in low densities or scattered distributions under good quality conditions can be/are included as indicator species. This is expected to be an important point in many cases as important indicator species are even under good quality conditions not expected to be present in each sample (especially in case of use of observation techniques with relatively small surfaces sampled). Assessing quality status at the level of single samples might overestimate the real quality status as it is expected to be crucial that a range of less common indicator species should be present at 'larger' scale under good quality conditions (e.g. to reflect complete benthic diversity and completeness of all expected ecological functions) , that cannot be replaced by the presence of one or a few of such species in the majority of the samples. (This strength is however also a weakness of the methodology; see chapter 8.2).

The BISI is filling the gap between traditional observation based (diversity) indicators and pressure based modelling. Besides providing an assessment of the overall quality status, the BISI identifies those pressures responsible for observed quality status or developments indicating consequences towards ecological functioning.

The assessment methodology is perfectly geared for assessment of measures or management to improve benthic habitat quality status when applied in a specific design as it provides the tools for statistical testing of potential quality differences as well.

The BISI covers each of the biotic characteristics of good quality situations and possible deterioration as indicated by the European Commission (2017) with regards the MSFD: "Typical species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species". These aspects are directly or indirectly covered by the indicator species selections and potential inclusion of different observation methodology data selective for different parts of the communities.

8.2 Weaknesses

As the methodology is in the first place developed to detect changes in the quality status between an initial situation (T0) and future assessments, the accurateness of the internal (realistic) reference is something that has to be confirmed in the near future by the results of recurrent applications. 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 assessment tool for future evaluation, by focussing on areas with effective management measures, possibly combining sample locations with detailed pressure mapping. 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 assessed than in others. The reliability of the internal reference is likely dependent of the monitoring efforts in the past (that show spatial differences).

The derivation of the internal reference is standardized as much as possible making assessments of different areas more comparable, although there is room for interpretation and 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 assessment results. Additionally a broader application in various regions, including possible identification of reference areas, might give opportunities for improvement of the comparability of assessments. Therefore recalculations of internal references as a calibration exercise are suggested for areas for which series of observation data 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. Normalisation at the level of countries or monitoring programmes might be a solution to overcome methodological differences in assessment result. 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, compared 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.

The methodology is meant/developed for benthic habitat quality assessment at the level of areas/habitats/ecotopes based on several (a sufficient number) representative samples, as important quality aspects include the presence of certain (indicator) species occurring in relative low densities or scattered spatial distributions under good quality conditions. Not including such species in the assessment (e.g. quality assessment based on a single sample with a small surface) likely overestimates the quality status (as only common and abundantly present species can be included). (Assessment at the level of areas is therefore also a strength of the methodology; see chapter 8.1). A drawback of quality assessment at the level of entire areas is however that there is less flexibility in assessing (reporting on) percentages of area in a certain quality state (which is however amongst others what is asked to be reported with regards to MSFD D6; European Commission, 2022; Raicevich, 2024).

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, can have some impact on assessment results. As the number of species under debate that are included in the BISI is kept to a minimum and the methodology is based on a large number of indicator species, it is expected that possible impact on assessment results is reduced. The BISI like other (for instance trait-based) indicators could benefit from international initiatives to align characterization and qualification of potential indicator species, i.e. by use of common sources and central storage of indicator characterisations (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 and where possible resolve possible weaknesses.

With regards to the MPAs, BHTs and HD habitat types of the Dutch part of the North Sea series of assessments and even trends based on BISI results have come available and are analysed and compared to disturbance patterns and conservation measures taken to improve benthic habitat quality. This provides confidence in the assessment results so far (Wijnhoven, 2023d). However, a specifically installed monitoring programme for BISI application (and benthic habitat quality assessment) is only in place since 2015 with recurrent sampling every 3 years. High power quality assessment of short to mid-long term developments can take place when about 5 observation moments (monitoring cycles) are available. Therefore testing and assessment opportunities for the BISI are still improving and seem to be especially informative when benthic community observation data are available for the monitoring series up and including 2027. Additionally, it is not until recent (March 2023) that the first (larger scaled) areas are closed for sea floor disturbing fisheries as a measure to achieve benthic habitat quality improvement. Although monitoring in these areas has been installed in and soon after 2015, it is just the coming years that we might expect real differentiation in benthic habitat quality status. These are good test cases for the BISI as well.

At the moment specific BISI assessment tools to report on the quality status and developments of marine habitat types with regards to 'structure and function' under the Habitats Directive are in development ($BISI_{S\&F}$), based on the BISIs available for marine HD habitat types so far (Wijnhoven, 2023a,b). In 2024 these $BISI_{S\&F}$ assessment tools will be applied to inform the Article 17 reporting under the HD in 2025. This will be the first application of the BISI for several HD areas and habitat types that include several years of observation.

Assessment tools for application at (sub) regional level at the level of BHTs have been developed for OSPAR region II (Greater North Sea region). During the developmental phase there has been some first smaller scale testing on international data around the Dogger Bank. The set of benthic community data collected for the QSR2023, which have already been through some standardization steps, for other indicator (BH2b; Wijnhoven et al., 2023) application seem to be perfect for a broad scale pilot study. Challenges might be how to deal with differences in observation methodologies (that have been shown to result in different results for other indicators in case no compensating steps are taken) and in data resolutions and monitoring designs. Beneficiary will however be that other indicators including pressure mapping has taken place and is available for interpretation from the QSR2023 reporting (OSPAR, 2023a). The BISI will therefore be suggested for an official pilot assessment under OSPAR (Biodiversity Committee) on the way to be forwarded as a candidate indicator with regards to Benthic Habitats in the OSPAR systematics. Within the BH assessment framework (Elliott et al., 2018) the BISI could fill the gap of indicating which pressures are responsible for impact on the observed quality to inform pressure based modelling (BH3, BH4), besides providing the benthic habitat quality status on basis of benthic community observation data where the combination with BH1 and BH2a,b could provide an accurate assessment under various condition (OSPAR, 2023b) (including multi-pressure situations and sub regions with lack of reference conditions).

BISIs developed for various situations represent living methodologies, which allow adjustments of used selections of indicator species, indicator values and reference values according to new insights. Although in that case recalculation of earlier assessments 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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