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INTERCALIBRATION REPORT FOR BENTHIC INVERTEBRATE FAUNA OF THE NORTH EAST ATLANTIC GEOGRAPHICAL INTERCALIBRATION GROUP FOR COASTAL WATERS, WADDENSEA TYPE (NEA 3/4)

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Intercalibration of biological elements for transitional and coastal water bodies.

North East Atlantic Geographical Intercalibration Group (NEA-GIG):
Coastal Waters, Wadden Sea type (NEA 3/4)– Benthic Invertebrate fauna

Contents

1 Summary

This report gives a technical description of the intercalibration of the two benthic multi-metric assessment approaches (BEQI2 and m-AMBI) for soft sediment habitats in the North East Atlantic Geographical Intercalibration Group (NEA-GIG) for type NEA 3/4 (Wadden Sea). Two European Member States (Germany, the Netherlands) are involved. This process is executed under the form of a JPI oceans pilot action. All information, in relation to indicator algorithm, boundary settings and references are summarized in this report. The two benthic assessment approaches consist both of the same metrics (parameters) and differ only in their EQR calculation algorithm. The BEQI2 has a fixed formula and a priori pooling of the samples, whereas the m-AMBI is based on a factor analysis. Both countries used the best available information (e.g. areas with least disturbed conditions) and their expert judgment to delineate appropriate reference datasets, to derive the reference values for each parameter from (e.g percentiles approach). The boundaries were slightly different between the Netherlands and Germany, and are respectively 0.6 and 0.7 for G/M and 0.8 and 0.85 for H/G.

Based on the available benthic data in the different habitats in the Dutch and German Wadden Sea, a common benthic dataset was constructed, focusing on the intertidal habitats. Only for this habitat type, a set of common benchmark data could be defined, subjected to a similar level of eutrophication and a very low fishery pressure.

The benthic assessment approaches of the Netherlands and Germany meet all WFD compliance criteria. Both approaches are well tested in relation to different types of pressures, in literature (Borja et al., 2009; Van Loon et al., 2015) and NEA-GIG coastal waters intercalibration report.

The comparability analyses reveal the following results. The regression comparison shows that both methods correlated very well (R^2= 0.9103). The boundary bias criteria are above 0.25 for the H/G boundary of the m-AMBI and G/M boundary of the BEQI 2. The H/G boundary of the m-AMBI is slightly above the criteria, but a change is not suggested by the comparability algorithm in the excel sheet. The G/M boundary of the BEQI2 need to be slightly increased to meet the boundary bias criteria by 0.11 to 0.611.

After consultation of both countries, the Netherlands has agreed to change the G/M boundary for type NEA3/4 into 0.61 to meet the comparability criteria.

2 Introduction

This report gives a technical description of the intercalibration of the two benthic multi-metric assessment approaches (BEQI2 and m-AMBI) for soft sediment habitats in the North East Atlantic Geographical Intercalibration Group (NEA-GIG) for type NEA 3/4 (Wadden Sea). Two European Member States (Germany, the Netherlands) are involved.

In the intercalibration phase I and II, no progress was made for the intercalibration of type NEA3/4, due to development issues of the indicators and priority to the intercalibration of the coastal and transitional water types. Therefore, in phase III, under the form of a JPI oceans pilot action (http://www.jpi-oceans.eu/intercalibration-eu-water-framework-directive), this process has been executed. The objectives of this action are:

- WFD method compliance documentation check, explanations of the justifications for assessment methods including specific parameters, reference conditions and the boundary setting procedure. Also to check the pressure-response relationships.
- Provide an alternative benchmarking clarification, trying to take regional biological differences and sampling protocol differences into account, based on already available data or validated expert judgment.
- Check and improve comparability analysis.
- Prepare and compile finalized intercalibration technical report.

This report compiles all the latest information regarding the benthic assessment approaches, boundary- and reference settings for each Member State and common dataset characteristics. The pressure-response relation of both assessment methods was already proved by previous analyses (Borja et al., 2009; Van Loon et al., 2015). Specific analyses were conducted to detect possible bio-geographical differences in the common dataset, perform an alternative benchmark delineation and the comparability analyses following the intercalibration guidelines (Guidance document 14: guidance document on the intercalibration process 2008-2011).

3 Description of national assessment methods

A benthic assessment approach consists of an indicator algorithm, boundary settings and a reference setting approach. Two benthic assessment approaches need to be intercalibrated in this case. The Netherlands used the BEQI2 method to evaluate the ecological status in type 3/4; whereas Germany selected the m-AMBI method.

3.1 Methods and required BQE parameters

The current intercalibration exercise is based on the latest versions of the multi-metric indicator algorithms (Table 1). The BEQI2 consist of the parameters species richness, Shannon wiener and AMBI and were equally weighted in the EQR determination (Van Loon et al., 2015). The m-AMBI takes into account the same parameters, but the EQR is determined based on a factor analysis (Borja et al., 2004; Muxika et al., 2007). The EQR values determined for the samples within the common dataset are re-calculated based on those algorithms. The benthic parameters (species richness, Shannon diversity and AMBI) for the multi-metric or multivariate analyses are derived from the AMBI tool.

The WFD requires the inclusion of certain metrics within the national assessment method for benthic invertebrates, which are summarized for each Member State in Table 2. Both assessment methods contain the required parameters.

Table 1. Overview of the algorithms of the two assessment methods. H': Shannon wiener diversity; S: Number of species; AMBI: AZTI Marine Biotic Index.

	MULTIMETRIC	
BEQI2 (The Netherlands)	$EQR (ecotope) = 1/3 * [Sass / Sref] + 1/3 * [H'ass / H'ref]^1 + 1/3 * [(6 – AMBI_{ass})/(6-AMBI_{ref})]$	Van Loon et al., 2015
	MULTIVARIATE	
M-AMBI (Germany)	Factor analysis: S, AMBI, Shannon diversity index[1]	(Borja et al., 2004 and Muxika et al., 2007) http://ambi.azti.es

[1]Shannon diversity: log base 2.

Table 2. Overview of the metrics included in the national assessment methods

Member state	Full BQE method	Taxonomic composition	Abundance	Disturbance sensitive taxa	Diversity	Bio-mass	Taxa indicative of pollution	Combination rule of metrics
Netherlands	Yes	Not strictly – only as groups (5) of different sensitivity	As relative abundance of different sensitivity groups and proportional abundance in Shannon Wiener index	5 sensitivity classes (AMBI)	Yes, number of species and Shannon Wiener index	No	Group of opportunistic species	Average of 3 univariately normalized indicator EQR scores
Germany	Yes	Not strictly – only as groups (5) of different sensitivity	As relative abundance of different sensitivity groups and proportional abundance in Shannon Wiener index	5 sensitivity classes (AMBI)	Yes, number of species and Shannon Wiener index	No	Group of opportunistic species	Factorial analyses, calculating vectorial distances to reference conditions

3.2 Sampling and data processing

The benthic sampling procedure for the WFD Monitoring within the Netherlands and Germany for type NEA 3/4 is slightly different, especially regarding the sampling design.

The benthic sampling in the intertidal habitats in Germany are done by cores (different sizes possible) at certain locations. At each location 10 replicate samples were taken. In the Netherlands transect sampling is applied. Each transect is composed of 10 (Balgzand) or 20 (Piet Scheveplaat) stations. At each station, 2 (Piet Scheveplaat from 2009 onwards), 3 (Piet Scheveplaat before 2009) or 5 (Balgzand) replicate small core samples have been sampled and combined. The sample area of the cores and the number of cores combined per station show some changes during the years, which is document in several monitoring reports of NIOZ and Koeman and Bijkerk, the external benthos laboratories.

The processing of the samples is similar, with identification and counting of the individuals to species level. The taxonomy in both countries is standardized regarding WORMS. The level of the species determination and truncation rules are country specific and applied on the entire data set.

3.3 National reference conditions

The determination of the reference conditions is a complicated subject (Van Hoey et al., 2010; Birk et al., 2013). The ecological status in the WFD has to be measured as a deviation from a reference condition. These reference conditions need to correspond to largely undisturbed (='near-pristine') conditions (no or minor impact from human activities). Indeed, the lack of appropriate reference sites or robust historical datasets is one of the major problems addressed in the intercalibration exercises and in setting the good ecological status boundaries (Borja et al., 2007; 2009). Scientists are faced with virtual lack of undisturbed sites along the European coasts and estuaries, and historical data are not easily accessible (Borja et al., 2004). Reference settings will need to be based on clear stressor-response relationships, a knowledge of the 'naturalness' of the system; and expert judgment may also have a role to play (Van Hoey et al., 2010). As summarized in Table 3, both countries used the best available information (e.g. areas with least disturbed conditions) and their expert judgment to delineate appropriate reference values for their metrics. For most methods, the principle is to use highest indicator value which is not an outlier. For this reason, high percentile values (99 to 95p) (for AMBI low percentile values; 1 to 5 p) are mainly used (Van Loon et al., 2015).

The reference values used to calculate the EQR values for each sample within a habitat (also referred to as ecotopes in the BEQI2 MMI) in the common dataset are listed in Table 4. Those values were applied per benthic assessment approach on the common dataset.

Table 3. Overview of the methodologies used to derive the reference conditions for the national assessment methods included in the IC exercise

Member State	Type and period of reference conditions	Number of reference sites	Location of reference sites	Reference criteria used for selection of reference sites
Germany	Expert knowledge, Historical data, Least Disturbed Conditions; reference time: 1959 up to now. Habitat-specific. The highest values from the reference data sets were selected as reference values for AMBI, Diversity and richness. As reference value for the bad conditions 0 is used for Richness and Diversity, 6 for AMBI.	Not true reference sites, but least disturbed sites, 6 sites for subtidal, 9 sites for littoral stations (two in the common intercalibration dataset.	different sites Wadden Sea of Lower Saxony	The communities at the sites had to correspond with description of the reference community description referring to a certain habitat. This approach is based on the hypothesis that most undisturbed areas are still found in small patches and will be represented by the best sites in the data set of the corresponding habitat.
Netherlands	(a) Historical data for 1991-2006; (b) Estimation of reference values: AMBI(ref): the 1 percentile value; S(ref) and H'(ref): 99 percentile of S and H' for dataset 1992-2006 (15 years). The principle is to use highest indicator value which is not an outlier. (c) theoretical bad values: S(bad) = 0; H'(bad) = 0; AMBI(bad) = 6. (c)	Not true reference sites, but least disturbed sites can be selected if necessary, primarily in the intertidal area Piet Scheveplaat, where the fishery is minimal.	The Piet Scheveplaat in the Wadden Sea is a reference site for intertidal habitat.	Not applicable because marine waters in The Netherlands are always subject to at least some level of anthropogenic impact. However, least disturbed samples from distinct sampling locations can be selected based on expert judgment using information on pressures at the sampling locations.

[1]Changed compared to the WISER input, based on Van Hoey et al., 2014 report.

Table 4. Overview of the reference values per benthic characteristics used in the intercalibration exercise.

Intertidal	Habitat	Sampled surface (m^2)	Sampling device	Species richness	Shannon (H' log2)	AMBI
Germany	Sand	0.2	plastic tubes	20	3.24	0.02
Germany	Muddy Sand	0.2	plastic tubes	21	3.11	1.61
Germany	mud	0.04	plastic tubes	20	2.9	2
Netherlands	muddy sand	0.1m^2	Manual cores (0,008m^2)	29	3.6	0.54

Subtidal	Habitat	Sampled surface (m²)	Sampling device	Species richness	Shannon (H' log2)	AMBI
Germany	Subtidal high dynamic (sand)	0.9	Van Veen	36	3.61	0.36
Germany	Subtidal low dynamic (muddy sand to sand)	0.9	Van Veen	30	3.77	0.05
Netherlands	Subtidal	0.12 (2 boxcores of 0.06 m^2 pooled)	Boxcorer	23	3.5	0.54

Two questions arose from analyzing this table:

1) The species richness between the muddy intertidal and other intertidal habitats in Germany, is not that different, despite the difference in sampling surface (0.04 compared to 0.2 respectively).

This estimation of the reference values is appropriate for this moment, because no differences in the number of species could be detected if the sampled area was enlarged. Therefore, the reference values for the intertidal mud for an area of 0.181m² can be considered as the same as for an area of 0.04m².

2) There is a difference between the reference values for the intertidal habitats of Germany and the intertidal habitat of the Netherlands. The values in the Netherlands were higher than in Germany, despite the lower sampling surface.

This difference in reference values, especially for species richness can be attributed to the following facts:

- The sampling design, which is point sampling (10 samples) in Germany and transect sampling (3*20 samples) per location in the Netherlands.
- The species richness in the Netherlands is also estimated based on pooling and aggregating samples over a wider spatial range (more than one location). This leads to relatively higher reference values for S (see Van Loon et al. 2015, Figure 3). In Germany it is location specific.
- And also some difference in the taxonomical truncation rules between the countries.

There is a big difference in total sampled area per country in the common dataset, which result in a different amount of species encountered in the data. For the intertidal muddy sand habitat, Germany founds 85 species (19 rare species), whereas the Netherlands 143 (40 rare species). This differences in species pool for both datasets, resulted from difference in total sampled area and sampling strategy, reasons for difference in reference values.

3.4 National boundary setting

The boundary setting procedure for both countries is summarized in Table 6. The boundary values used in the intercalibration for Germany and the Netherlands for type NEA3/4 were summarized in Table 5.

Table 5. The boundary values (High/good and Good/moderate) for the different assessment approaches as used in the intercalibration exercise.

	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
Germany	0.85	0.70	0.40	0.20
Netherlands	0.80	0.60	0.40	0.20

Table 6. Explanations for national boundary setting of the national methods included in the IC exercise

Member State	Type of boundary setting	Specific approach for H/G boundary	Specific approach for G/M boundary	BSP: method tested against pressure
Germany	Boundaries taken over from the intercalibration exercise (Borja et al., 2007[1]). Calibrated against pre-classified sampling sites. The boundary setting procedure is in line with the WFD's normative definitions.			The boundaries were additionally adjusted by the assessment of expert judgment (Heyer 2007). The m-AMBI relates to pressures of sediment enrichment, eutrophication and hazardous substances (Muxika et al. 2007).
Netherlands		The Good/Moderate boundary of 0.60 is primarily derived from the initial G/M boundary for sheltered coastal waters (Van Hoey et al., 2015), which was estimated using expert judgment and set at 0.60 (see. Van Loon et al. 2015, paragraph 2.7. for more information).		

3.5 Results of WFD compliance checking

Table 7. WFD compliance checking criteria.

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes, for both benthic assessment approaches
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes, for both benthic assessment approaches (see Table 12 and Table 6).
3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole.	The two Member States included the relevant parameters (see Table 2), A combination rule to combine parameter assessment is defined by both benthic assessment approaches.
4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT	Yes, for both Member States
5. The water body is assessed against **type-specific near-natural reference conditions**	No (see Table 3). Alternative benchmark conditions (based on a "least disturbed condition" criteria) had to be defined due to the absence of near-natural reference conditions in the intercalibrated type.
6. Assessment results are expressed as **EQRs**	Yes, for both benthic assessment approaches (Table 5).
7. Sampling procedure allows for **represent-tative** information about water body quality/ecological status **in space and time**	In most cases, the monitoring is considered as representative by the Member State itself. This aspect is not confirmed by specific, standardized analyses to test their representativeness. Sampling procedures are outlined in general, but not linked with the running WFD monitoring programs.
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes, for both benthic assessment approaches. The sampling procedure defined by each Member State allows the collection of species-abundance data, which is necessary to calculate all metrics of the different benthic assessment approaches.
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes, for both benthic assessment approaches, with some difference in taxonomic detail per Member State, but sufficient comparability. The taxonomic discrimination rules are country species and applied to each member states dataset.

There can be concluded that all compliance criteria were met for both benthic assessment approaches.

3.6 Typology

In the NE Atlantic, seven basic intercalibration types have been agreed upon. In this report the type NEA3/4 is taken into account (see outline of characteristics in Table 8).

Table 8. NEA GIG Intercalibration Type NEA3/4

New Type ID	*Name*	*Salinity [PSU]*	*Tidal range (m)*	*Depth (m)*	*Current velocity (knots) [m/s]*	*Exposure*	*Mixing*	*Residence time*
CW – NEA3/4	Polyhaline, exposed or moderately exposed (Wadden Sea type)	Polyhaline (18 - 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (0,51-1,54m/s)	Exposed or moderately exposed	Fully mixed	Days

This type is only discriminated in the Netherlands and Germany.

3.7 Pressures addressed

The BEQI2 and m-AMBI assessment approach are well tested against a pressure gradient. This pressure-response relation of both approaches are published in literature (Borja et al., 2009; Van Loon et al., 2015) and intercalibration report (NEA-GIG coastal waters, Van Hoey et al., 2015). Both methods are sensitive to various types of pressures, as eutrophication, oxygen depletion (see Dutch example), physical disturbance (see German sand extraction example) and increased suspended matter (see Dutch example).

Dutch example (Van Loon et al., 2015):

The sensitivity of the BEQI2 for human and natural induced stressors was explored by regression analysis of regional BEQI2 and time-series of measurements of dissolved oxygen in the Westerschelde mesohaline-intertidal ecotope and of the suspended matter concentration in the Dollard mesohaline-intertidal ecotope (Figure 1). The BEQI2 shows a positive, significant correlation with oxygen concentration, meaning that an increase in oxygen concentration leads to a higher BEQI2 EQR. Beside it, the BEQI2 shows a negative, significant correlation with suspended matter, meaning that a higher SPM concentration leads to a lower BEQI2 EQR.

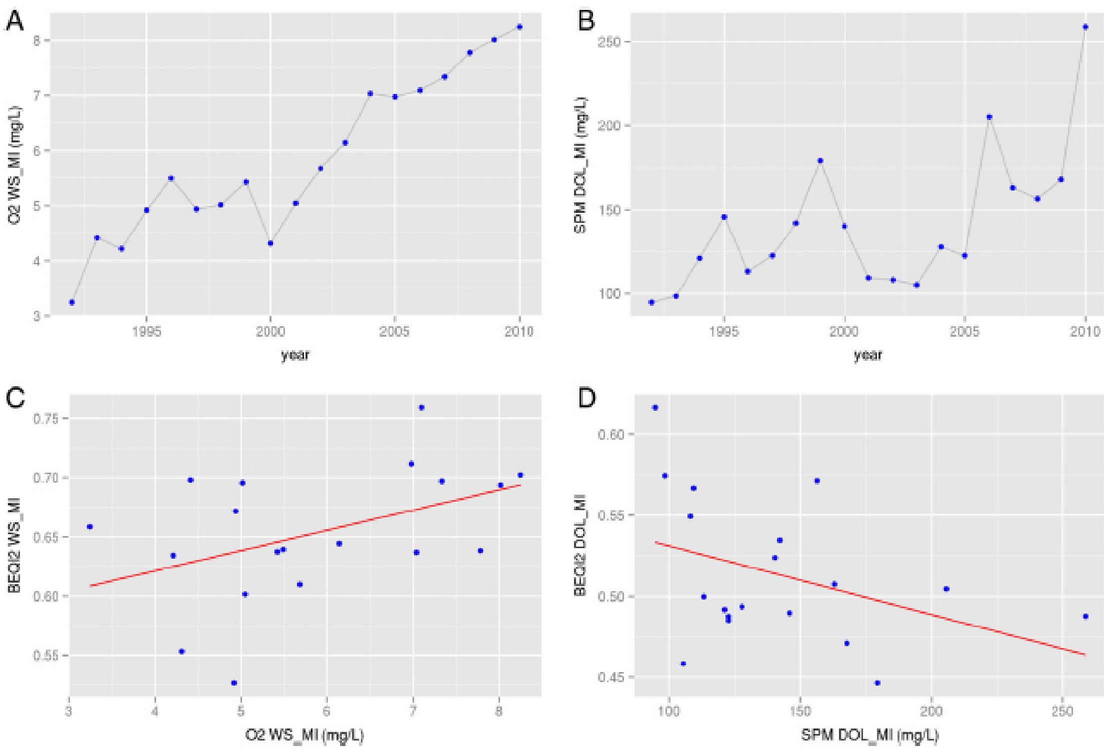

Figure 1. A-B Time trends of the state parameters oxygen (mg O2/L.year) and suspended matter (mg SPM/L.year) in the waterbody ecotopes Westerschelde mesohaline-intertidal (WS_MI) and Dollard mesohaline-intertidal (DOI_MI), respectively. C-D. State-impact correlations for oxygen concentrations and suspended matter with BEQI2 EQRs in the waterbody ecotopes Westerschelde mesohaline-intertidal (WS_MI) and Dollard mesohaline-intertidal (DOI_MI), respectively.

German example:

In the Dangaster Außentief (German Wadden Sea) in July 1996 huge sand extraction (1,2 million m^3 sand) took place. Before (June 1996) and after sand extraction the macrozoobenthos was investigated at several stations (Fischer et al. 2004) twice or thrice a year (April, June and September) until June 2000. With the data of five (E4, E5, E7, E11 and E17) out of these stations the M-AMBI values were calculated (Figure 2). The chosen stations laid to the south and in a distance between 50 m to 300 m from of the sand extraction area.

The M-AMBIs were calculated with the NL reference values given by (van Hoey et al. 2007) (AMBI 0.6, diversity 2.35 and richness 24). It is a static and correlative comparison, as no specific pressure linked variable (as organic matter content, sediment re-suspension or suspended matter), is available.

The ecological status decreased from a 'good' ('II') to a 'moderate' ('III') (Figure 2). In September 2000 the M-AMBI increased again.

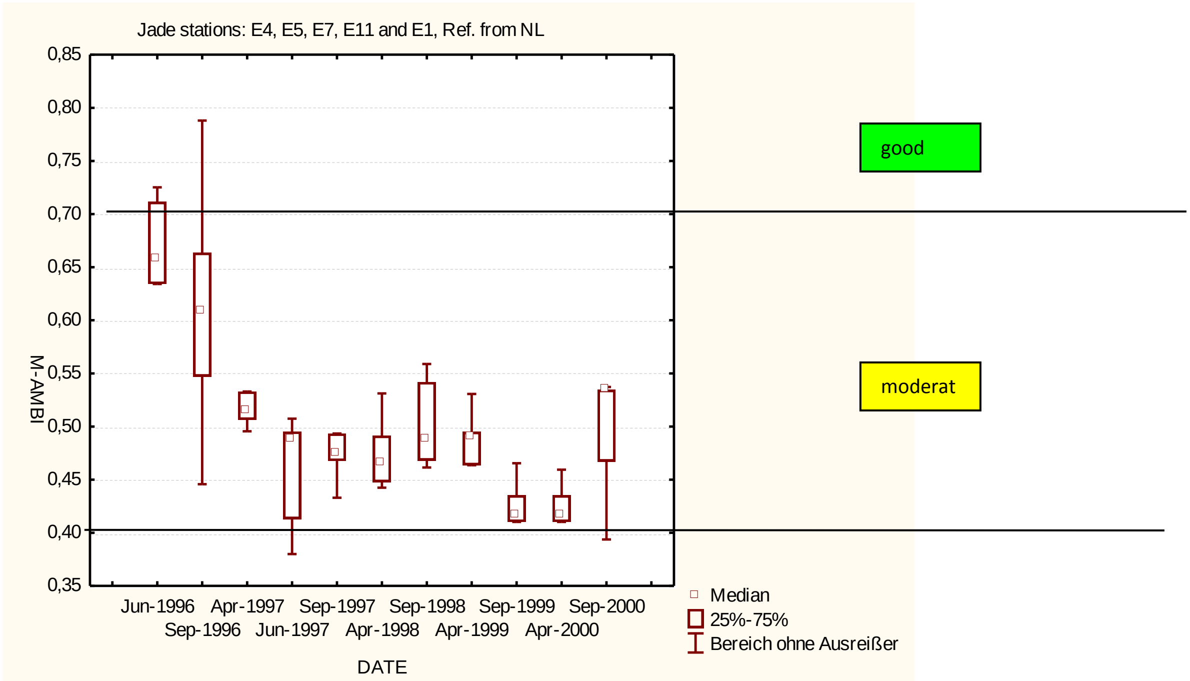

Figure 2. M-AMBI values at each sampling data in the BACI design monitoring for sand extraction at Dangaster Außentief.

3.8 Assessment concept

Do all national methods follow a similar assessment concept?

The two benthic assessment approaches for type NEA3/4 are very similar. They consist both of the same metrics (parameters) and differ only in their EQR calculation algorithm. The BEQI2 has a fixed formula and a priori pooling of the samples, whereas the m-AMBI is based on a factor analysis.

The main difference in assessment concept between the Netherlands and Germany is situated in how the raw data is pooled for determining the EQR values per habitat type. The BEQI2 assessment approach executed a randomisation procedure, which pool the small core samples obtained within a single habitat-year at random to 0.1m² (sample pool size) and repeat this 10 times to calculate per habitat the average BEQI2 score. This lead to an EQR value per year for each habitat within a waterbody. The Germany assessment approach pool the core samples per station a priori to the calculation of the EQR values for that station by the m-AMBI. The number of samples can vary between station and habitat type. If more stations are available per habitat type/waterbody, those EQR values need to be 'averaged' to come to an EQR value per habitat within a waterbody. For both assessment methods, the reference values were in accordance with the pooling principle and obtained sample pool sizes (see Table 4).

Due to this situation, we have different levels (habitat versus location) and sampling areas between both assessment approaches to calculate the EQR values. Therefore, this difference in concept is harmonized for intercalibration purpose. It is clear that it is not appropriate to calculate the EQR values on sample level (core or grab), due to the fact that both countries do it on a higher level (standardised sample pool surface). Therefore, we decided to work with a 'common' fixed sample size of 0.81m^2 for the intercalibration, which is the standard for the German assessment approach, but not in correspondence with the Dutch assessment way. For harmonization purpose, the data of the Netherlands is split in separate location assessments instead of an entire habitat assessment. This is feasible and acceptable and the relation between both approaches should be more or less the same, regardless the level of pooling.

	BEQI2	m-AMBI
Dutch dataset	A priori pooling of the subsamples to corresponding sample pool size of the Dutch reference values.	A priori pooling of the subsamples to corresponding sample size of the German reference values. By this the German reference values can be used for the assessment of the Dutch data.
German dataset	BEQI2 calculated on the a priori pooled German subsamples. The BEQI reference values can be used, despite their is a slight difference in total sample surface.	A priori pooled subsamples (10) to corresponding surface per location, as the German assessment method is.

In this case, we have compared 143 (German dataset) and 180 (Dutch dataset) sample assessments, which should give enough values to test the comparability criteria (Table 9). This create an unequal balance in data between both countries, but this has no influence on the comparison results. If the data of the years 2000 and 2001 in the Dutch dataset were not considered, the same results were obtained regarding the boundary adjustment (from 0.6 to 0.611).

Is the Intercalibration feasible in terms of **assessment concepts?**
Yes, despite some small difference in the way the EQR calculation occur for both benthic indicator approaches.

4 Collection of intercalibration dataset and benchmarking

4.1 Dataset description

At the start of the project, we had an expert meeting where we discussed the data availability and appropriateness. First, we decided to use autumn data only, to exclude seasonal variation. Second, we decided to focus on intertidal habitats, because most appropriate intercalibration data could be derived for it. This in the light of selecting benchmark samples. For the subtidal habitats, no appropriate pressure data was available, neither sites could be selected as benchmark sites by expert judgment. For the intertidal habitats, sites for both countries with similar level of eutrophication and negligible fishery pressure could be selected. Finally, the benthic data from the muddy sand habitat in the intertidal was selected because the Dutch monitoring focused on this habitat type and also a lot of German sites belong to this habitat type (Table 9). The similarity in the samples of the Netherlands and German for the intertidal habitats is investigated in section 4.2.1 (Multivariate analyses) and is very good.

Therefore, due the availability of benchmark sites for the intertidal muddy sand in both countries and a large amount of data, the comparability of the assessment approaches is tested on this data set.

Table 9. Overview of the available data and its metadata information

Dataset	Station	program	#assessment	Time perio	Grouping of subsamples	Total surfa	Waterbody type	Habitat/ecotoop	Benchmar
GE1	AuWe_MZB_3	NLWKN	8	2007-2014	10*0,0181	0,181	N4_4900_01	intertidal sand	no
GE1	Nney_MZB_1	NLWKN	8	2007-2014	10*0,0181	0,181	N4_3100_01	intertidal sand	no
GE1	Nney_MZB_2	NLWKN	8	2007-2014	10*0,0181	0,181	N4_3100_01	intertidal sand	yes
GE1	Nney_MZB_3	NLWKN	8	2007-2014	10*0,0181	0,181	N4_3100_01	intertidal muddy sand	no
GE1	Nney_MZB_5	NLWKN	8	2007-2014	10*0,0181	0,181	N4_3100_01	intertidal mud	no
GE1	Nney_MZB_6	NLWKN	8	2007-2014	10*0,0181	0,181	N4_3100_01	intertidal mud	no
GE1	Nney_MZB_7	NLWKN	8	2007-2014	10*0,0181	0,181	N4_3100_01	intertidal mud	no
GE1	Nney_MZB_8	NLWKN	8	2007-2014	10*0,0181	0,181	N4_3100_01	intertidal muddy sand	yes
GE1	WuKu_MZB_6	NLWKN	8	2007-2014	10*0,0181	0,181	N4_4900_02	intertidal muddy sand	no
GE1	WuKu_MZB_10	NLWKN	1	2007	10*0,0181	0,181	N4_5900_01	intertidal muddy sand	no
GE2	HH T1	HH	14	2000-2013	75*0,00166	0,1245		intertidal muddy sand	no
GE2	HH T2	HH	14	2000-2013	75*0,00166	0,1245		intertidal muddy sand	no
GE2	HH T3	HH	14	2000-2013	75*0,00166	0,1245		intertidal muddy sand	no
GE2	HH T4	HH	14	2000-2013	75*0,00166	0,1245		intertidal muddy sand	no
GE2	HH T5	HH	14	2000-2013	75*0,00166	0,1245		intertidal muddy sand	no
NL1	Balgzand-Raai J_A	Balgzand	15	2000-2014	substaal 1-12 (12*0,0157)	0,1884	Waddensea	intertidal mud-muddy s	no
NL1	Balgzand-Raai J_B	Balgzand	15	2000-2014	substaal 13-24	0,1884	Waddensea	intertidal muddy sand	no
NL1	Balgzand-Raai B_A	Balgzand	15	2000-2014	substaal 1-12	0,1884	Waddensea	intertidal muddy sand	no
NL1	Balgzand-Raai B_B	Balgzand	15	2000-2014	substaal 13-24	0,1884	Waddensea	intertidal muddy sand	no
NL1	Balgzand-Raai C_A	Balgzand	15	2000-2014	substaal 1-12	0,1884	Waddensea	intertidal muddy sand	no
NL1	Balgzand-Raai C_B	Balgzand	15	2000-2014	substaal 13-24	0,1884	Waddensea	intertidal muddy sand	no
NL2	Piet Scheveplaat - Raai 600_A	Piet Schev	15	2000-2014	substaal 1-10 (10*0,0157)	0,157	Waddensea	intertidal muddy sand	yes
NL2	Piet Scheveplaat - Raai 600_B	Piet Schev	15	2000-2014	substaal 11-20	0,157	Waddensea	intertidal muddy sand	yes
NL2	Piet Scheveplaat - Raai 601_A	Piet Schev	15	2000-2014	substaal 1-10	0,157	Waddensea	intertidal muddy sand	yes
NL2	Piet Scheveplaat - Raai 601_B	Piet Schev	15	2000-2014	substaal 11-20	0,157	Waddensea	intertidal muddy sand	yes
NL2	Piet Scheveplaat - Raai 602_A	Piet Schev	15	2000-2014	substaal 1-10	0,157	Waddensea	intertidal muddy sand	yes
NL2	Piet Scheveplaat - Raai 602_B	Piet Schev	15	2000-2014	substaal 11-20	0,157	Waddensea	intertidal muddy sand	yes

4.2 Data acceptance criteria

The Netherlands and Germany have delivered data for the intercalibration exercise.

To explore the common intercalibration dataset for benthic macro-invertebrates, we performed some standard multivariate analyses. This to evaluate the following aspects:

- to check for outliers (samples very different from the rest and showing a problem)
- If there were regional or sub-regional differences between the samples and habitats
- If different benthic communities could be detected, which can be related to different physical habitats (sedimentology).
- If there is any pattern in the data that justifies the delineation of sub-types for benchmarking, even the fact that we already select common types.

4.2.1 General multivariate analyses

For the purpose of the multivariate analyses, the common dataset is fourth root transformed to reduce the effect of very abundant species on the overall pattern. Beside this, the rare species (with less than 3 individuals) were excluded from these analyses to reduce the effect of rare species on the overall pattern. The similarity between samples is determined by the Bray-Curtis similarity. The sample groups were determined based on a cluster analyses, with cut-off level at certain similarity level (31). Multidimensional scaling (MDS) is used to visualize the cluster groups (Figure 3). The sample groups discriminated from the cluster analyses were compared with the habitat type considered by the experts (Figure 4).The analyses were executed in PRIMER6.

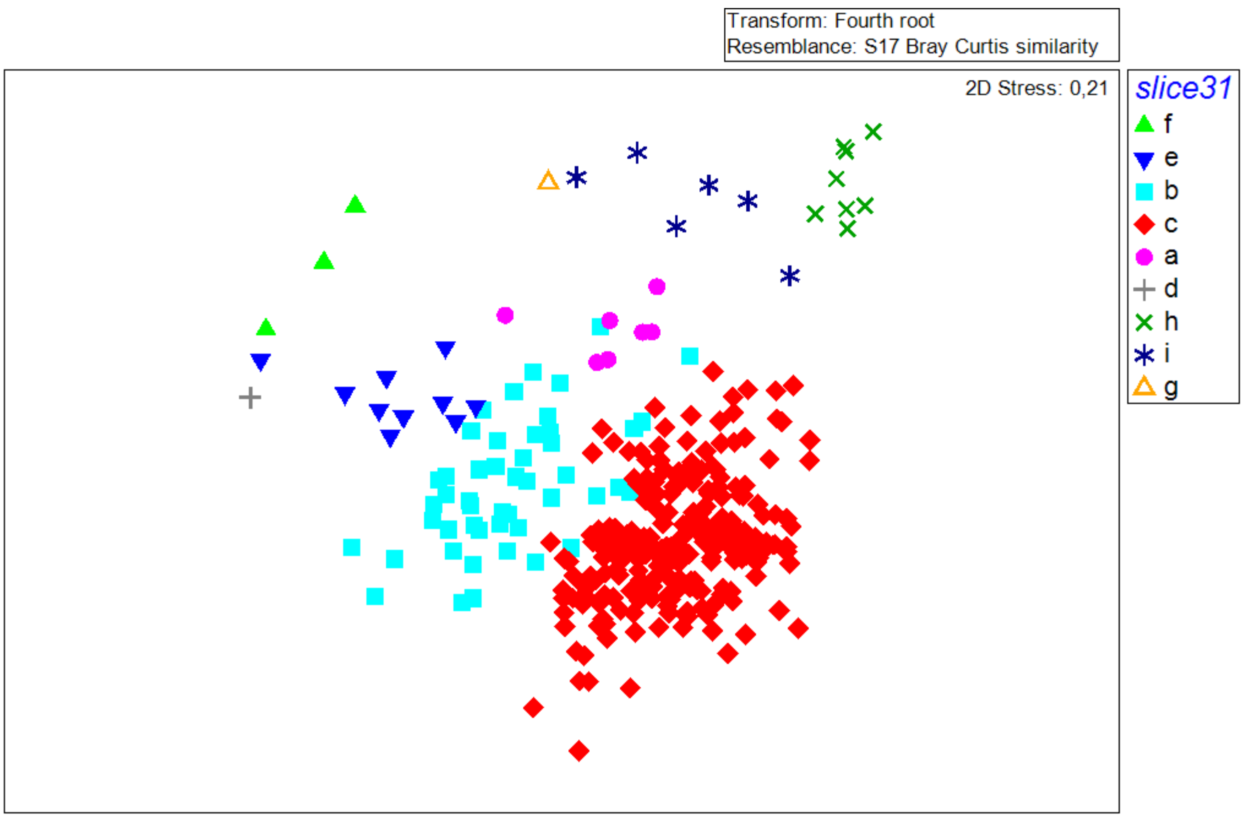

Figure 3. MDS of the cluster groups (slice 31 Bray Curtis similarity), which result in 9 groups and are coded alphabetically (a-i).

Some explanation on the cluster groups:

- No outlier samples present in the common dataset (no very different sample from the rest).
- The subtidal habitats clearly separated from the intertidal habitats, both in the cluster groups (a, e, f) as by the habitat groups (subtidal mud and fine sand). Those were not further considered for the intercalibration, because the focus is on the intertidal habitats.
- The intertidal mud habitat (Germany) clearly clustered separately from the others, in cluster i, g and h (location dependent). This means, that this habitat type could be a separated sub-type for the Wadden sea. Due to the absence of Dutch data for this type, this is not further considered.

- The samples, considered located in an intertidal sand habitat, could not be discriminated from the intertidal muddy sand habitat in the cluster analyses (belong to cluster b and c). This can mean that the location considered as intertidal sand, should not be a separate subtype for this intercalibration.
- The majority of the samples in the common dataset were from the intertidal muddy sand habitat and clustered together in two main clusters (b and c).
  - Cluster b contains the samples of Balgzand 'raai' ZDJ en AuWe-MZB3 and are slightly different from the other intertidal muddy sand locations.
  - Cluster c contains the majority of the samples and are reflecting the species composition of an intertidal muddy sand habitat in the Wadden Sea area. This cluster clearly groups the samples of this habitat type of both countries.

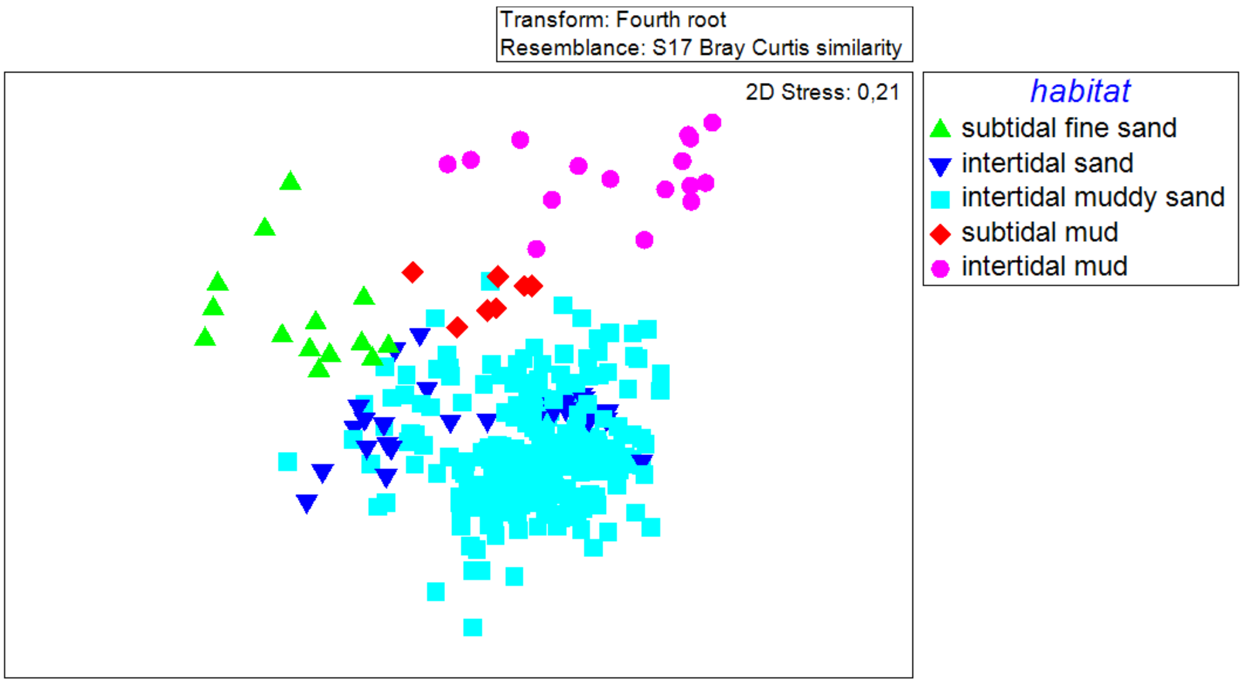

Figure 4. MDS with indication of the habitat types.

We can have concluded, based on the species composition, that the benthic fauna in the Wadden Sea area is similar between Germany and the Netherlands. There is no geographical difference in species composition and main characteristics within the intertidal muddy sand habitat type. This analyses also shows that it is relevant to consider the habitats separately, as sub-types if necessary. This means that it is preferred that the reference conditions are habitat specific, as Germany does. Only, the difference in community characteristics between intertidal sand and muddy sand is not obvious, due to the position of the intertidal sand samples in the MDS.

For the intercalibration exercise, we can clearly use the samples of the intertidal muddy sand habitat of both countries to test the comparability between both benthic assessment approaches.

4.3 Common benchmark

Both countries have select a benchmark site, that is subjected to a similar level of eutrophication, but consider the lowest influence of fishery. Details on the level of eutrophication and fishery for the German locations are given in the table in annex 1. Both pressures are the main driver for changes in the benthic system within the Wadden Sea area.

For the Netherlands this is the Piet Scheveplaat for the intertidal habitat and for Germany that is the Nney_MZ8 site for the intertidal muddy sand habitat.

4.4 Benchmark standardization

The principal aim of benchmarking in intercalibration is to identify and remove differences among national assessment methods that are not caused by anthropogenic pressure but rather by systematic discrepancies (due to different methodology, biogeography, typology etc.; see remarks in section 3.3 on reference settings) (Annex V, IC Guidance).

Benchmark standardization will correct for differences in median EQR values between the Member States' benchmark sites obtained by certain assessment approaches. Those median values will be corrected by the benchmark standardization procedure; this correction will be more obvious for cases where the medians are significantly different.

We tested whether benchmark standardization was necessary. Student's sT was used to compare the benchmark sites values for the two national methods.

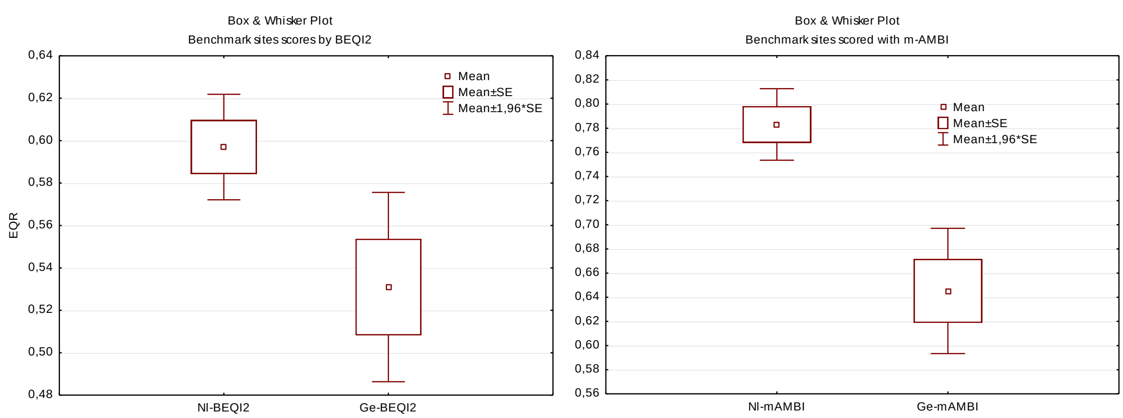

Figure 5. Box-whisker plot of the assessment of the Dutch and German benchmark sites with each benthic assessment approach.

The benchmark sites of both countries were not significantly different from each other for the BEQI2 (p = 0,155) (left box whisker plot) (Figure 5), despite the difference in the box plot. The benchmark sites of both countries were significant different with the m-AMBI approach (p = 0.0135) (right box-whisker plot) (Figure 5). This indicated that benchmark standardization is necessary.

The correlation between the average value of all national EQRs per survey in the full dataset was not significantly correlated with its standard deviation, therefore national EQRs does not converge towards the bad end of the quality gradient, and therefore, subtraction was used for the standardization.

5 Comparison of methods and boundaries

5.1 Intercalibration option and common metrics

Option 3a. Intercalibration can be performed based on commonly assessed sites and whether the ecological quality gradient is sufficiently covered. Only two methods are involved in the intercalibration, which involve that there is a direct comparison (pseudo-metric=other method).

5.2 Results of the regression comparison

The regression comparison shows that both methods correlated very well (R^2= 0.9103).

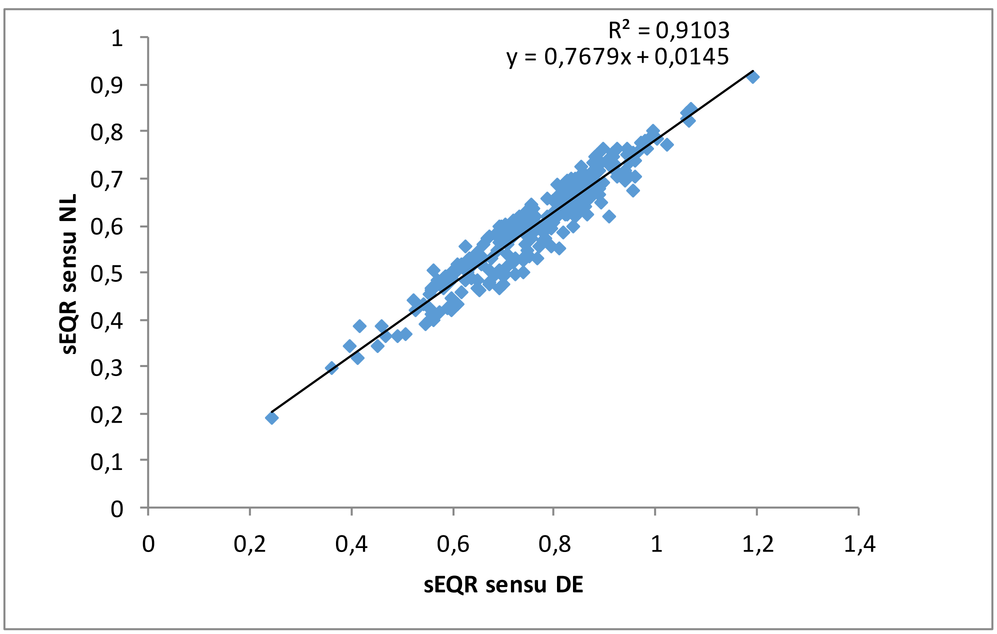

Figure 6. Scatter plot of EQR values of Germany and Netherlands, with linear regression line.

5.3 Comparability criteria

The boundary bias criteria are above 0.25 for the H/G boundary of the m-AMBI and G/M boundary of the BEQI2. The H/G boundary of the m-AMBI is slightly above the criteria, but a change is not suggested by the excel sheet. The G/M boundary of the BEQI2 need to be slightly increased to meet the boundary bias criteria by 0.11 to 0.611.

Table 10. Boundary bias values for the high/good and good/moderate boundary for the German and Dutch benthic assessment methods.

Boundary	A Germany	A on scale of B	B Nether-lands	B on scale of A	A average bias	B average bias	A excess as classes	A harmonised boundary	B excess as classes	B harmonised boundary
MP	0,400	0,415	0,400	0,400						
GM	0,700	0,715	0,600	0,582	0,194	-0,306		no change	0	0,611
HG	0,850	0,865	0,800	0,764	0,252	-0,200	0,002	0,850		no change

The average absolute class difference for the five classes between both methods is 0,35 (<0.5). If the poor and bad classes are not taken into account, the average absolute class difference is 0,39 (<0.5).

These results seem to be logically, because the boundaries for Germany are higher than for the Netherlands, but for the reference values it is the reverse. This lead to the fact that both benthic assessment approaches are comparable.

6 Final results to be included in the EC

6.1 Table with EQRs

A boundary adjustment for the G/M boundary by the Netherlands is needed. They accepted to increase the boundary to 0,61. The final boundaries for the benthic assessment approaches (BEQI2 and m-AMBI) for the Wadden Sea in the North-east Atlantic were given in Table 11.

Table 11. Boundary values of the different benthic assessment approaches after intercalibration. The boundaries in red are those changed after boundary harmonization.

		Ecological quality ratios			
Country	Benthic assessment approach	High-good boundary	Good-moderate boundary	Moderate-poor boundary	Poor-bad boundary
Germany	m-AMBI	0.85	0.70	0.4	0.2
Netherlands	BEQI2	0.80	0.61	0.4	0.2

6.2 Correspondence common types versus national types

The common type (NEA3/4) is recognized as type in every Member State and is related to the national types.

6.3 Gaps of the current intercalibration

Not all habitat types within the Wadden Sea could be considered, due to the absence of a comparable dataset for those habitats between both countries, especially in the light of discriminating appropriate benchmark sites for those habitats.

7 Ecological characteristics

7.1 Description of reference or alternative benchmark communities

The description of the benthic community characteristics at reference or alternative benchmark is summarized in Table 12. This information is generated from the WISER database. Only for France, Norway and Spain (Andalusia) this information is not available.

7.2 Description of good status communities

The description of the benthic community characteristics at good status is summarized in Table 12. This information is generated from the WISER database.

Table 12. Overview of the description by the Member States of the macro-invertebrate reference community and good status community

<table>
<tr><th>Member State</th><th>Description of reference community</th><th>Description of good status community</th></tr>
<tr><td>Germany</td><td rowspan="2">Benthic communities, species numbers, diversity typically for the habitat (sediment, salinity, exposure)- low number of opportunistic species.</td><td rowspan="2">High portion of sensitive taxa, complex communities, low number of opportunists, high species number and high diversity assemblages.</td></tr>
<tr><td>Netherlands</td></tr>
</table>

8 References

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Annex 1:

Table with the pressure info per location for Germany (** DIN = arithmetic mean of DIN winter means (Nov-Feb) - (from nearest monitoring point to MZB station).

					HABITAT			PRESSURES[2]					Eutrophication (DIN)			Fishery: ICES fishery map (indirect linking)			Benchmark sites
	Dataset name	Name of German authority responsible for the Data	German station Name	Water body type NEA 3 or 4	habitat/ecotope	Depth	Sediment	Pressure	quantitative	qualitative	expert judgment	remarks	Eutro/ high (DIN)	Eutro/m edium (DIN)	Eutro/ low (DIN)	Fishery/ high	Fishery/ medium	Fishery/ low	
1	German Wadden Sea	NLWKN	Bork_MZB_8	3	subtidal finesand	>6m	Finesand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011	1,25			high			
2	German Wadden Sea	NLWKN	AuWe_MZB_1	3	subtidal finesand	>6m	Finesand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011		0,47			medium		
3	German Wadden Sea	Bfg	Weser-4	3	subtidal sand	9m	Sand	eutrophication and fisheries		fisheries	yes					high			
4	German Wadden Sea	Bfg	Elbe-4	3	subtidal sand	12-15m	Sand	eutrophication and fisheries		fisheries	yes					high			
5	German Wadden Sea	Bfg	Elbe-5	3	subtidal sand	12-15m	Sand	eutrophication and fisheries		fisheries	yes					high			
6	German Wadden Sea	Bfg	Ems-4	3	subtidal sand with mud	9m	Sand with Mud	eutrophication and fisheries		fisheries	yes					high			
8	German Wadden Sea	NLWKN	Nney_MZB_6	4	litoral mud	intertidal	mud	eutrophication and fisheries	DIN	fisheries	yes	DIN 2007-2013	0,82			high			
9	German Wadden Sea	NLWKN	Nney_MZB_7	4	litoral mud	intertidal	mud	eutrophication and fisheries	DIN	fisheries	yes	DIN 2007-2013	0,82			high			
10	German Wadden Sea	NLWKN	Nney_MZB_5	4	litoral mud	intertidal	mud	eutrophication and fisheries	DIN	fisheries	yes	DIN 2007-2013	0,82					low	
11	German Wadden Sea	NLWKN	Nney_MZB_1	4	litoral sand	intertidal	sand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2007-2013	0,82			high			
12	German Wadden Sea	NLWKN	Nney_MZB_2***	4	litoral sand	intertidal	sand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2007-2013	0,82					low	yes
13	German Wadden Sea	NLWKN	Nney_MZB_3	4	litoral muddy sand	intertidal	muddy sand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2007-2013	0,82			high			
14	German Wadden Sea	NLWKN	Nney_MZB_8***	4	litoral muddy sand	intertidal	muddy sand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2007-2013	0,82					low	yes
15	German Wadden Sea	NLWKN	WuKu_MZB_6	4	litoral muddy sand	intertidal	muddy sand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2010	0,96			high			
16	German Wadden Sea	NLWKN	Bork_MZB_4	4	subtidal mud	<6m	mud	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011	1,25			high			
17	German Wadden Sea	NLWKN	AuWe_MZB_3	4	litoral sand	intertidal	sand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011		0,47		high			
18	German Wadden Sea	NLWKN	WuKu_MZB_10	4	litoral finesand	intertidal	finesand	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2010	0,96			high			
19	German Wadden Sea	BSU HH	HH T1	4	litoral sandy to muddy	intertidal	sandy to muddy	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011		0,58		high			
20	German Wadden Sea	BSU HH	HH T2	4	litoral sandy to muddy	intertidal	sandy to muddy	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011		0,58		high			
21	German Wadden Sea	BSU HH	HH T3	4	litoral sandy to muddy	intertidal	sandy to muddy	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011		0,58		high			
22	German Wadden Sea	BSU HH	HH T4	4	litoral sandy to muddy	intertidal	sandy to muddy	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011		0,58		high			
23	German Wadden Sea	BSU HH	HH T5	4	litoral sandy to muddy	intertidal	sandy to muddy	eutrophication and fisheries	DIN	fisheries	yes	DIN 2001-2011		0,58		high			

Table with the pressure info for the Dutch Wadden Sea

Column header	Specifications
Dataset name	Dutch Wadden Sea
Data owner	Rijkswaterstaat
Station names	Balgzand (Western Dutch Wadden Sea; 3 transects: B, C, J) Piet Scheveplaat (Eastern Dutch Wadden Sea 3 transects; 600, 601, 602)
NEA type	3-4 (Gert, can this be discriminated for the Western and Eastern part of the Dutch Wadden Sea?)
Habitat/ecotope	Litoral muddy sand
Depth	Intertidal
Sediment type	Muddy sand
Common pressure types	Eutrophication, fisheries
Pressures characterization method	Eutrophication: using NH4+NO2 results from QSR report Wadden Sea 2009, Thematic report No. 9 Eutrophication, Table 5. Fisheries: using QSR report Wadden, Thematic report No. 3.3 Fisheries, Figure 3.3.6 (shrimp fisheries), Figure 3.3.3 (Mussel seed fisheries).
Pressure data period	Eutrophication: 2000-2006 (QSR Wadden Sea) Fisheries: depends on fishing type, around 2000-2007.
Pressure quantification	Eutrophication: in the Western Dutch Wadden Sea, the assessment value (period 2000-2006) of 8.2 uM NH4+NO2 is just below the "problem condition limit" of 8.3 uM. In the Eastern Dutch Wadden Sea, the assessment value of 16.8 uM (period 2000-2006) exceed the problem condition limit of 10.2 uM. In conclusion, there is significant eutrophication in the Dutch Wadden Sea, especially in the Eastern part. Note however that for benthos, some amount of eutrophication is probably not a problem, because it delivers additional food for filter feeders. Fisheries: 1. Shrimp fisheries only occurs in subtidal parts, mainly in the Western Wadden Sea**. No shrimp fisheries** occur in the **intertidal areas** because these areas are too shallow for fishing boats. 2. Mussel seed fisheries mainly occur in the subtidal areas in the Western Wadden Sea, and **not in the intertidal parts.** 3. Since January 2005 mechanical cockle fishery in the Dutch part of the Wadden Sea is not allowed any longer. Only manual cockle fishery is still allowed with a maximum yearly catch of 5% ofthe cockle stock. The fished amounts were between 0.1 and 1.5 % of the stock. So there is some manual cockle fisheries in the intertidal parts of the Wadden Sea, but this pressure is probably relatively low. In conclusion, the **fisheries pressure** in **the intertidal parts** of the Dutch Wadden Sea is **low**. In the subtidal parts, especially of the Western Dutch Wadden Sea, the fishing pressure is relatively high.
Benchmark sites	Yes, Piet Scheveplaat.

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