Assessment of marine benthic quality change in gradients of disturbance:

Comparison of different Scandinavian multi-metric
indices

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Preface

This report is a product from the project HARMEX “Harmonisering af Nordiske bundfaunaindeks for økologisk kvalitet i poly- til eu-haline områder”, which is an RTD project founded by the Nordic Council of Ministers, involving participants from Sweden, Norway and Denmark.

The project was inspired by the EU Geographical Intercalibration Work (GIG) for the implementation of the Water Framework Directive (WFD) and may be regarded as an extension of that work in order to harmonise specifically Scandinavian methods for the assessment of benthic macrofaunal ecological quality. Particular focus has been paid to developing alternative methods to estimate the border between *Good* and *Moderate* Ecological Quality Status, the so called G/M border.

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Summary

In this study we compared three Scandinavian multi-metric indices to assess marine coastal benthic ecological status in seven different gradients of disturbance related to pollution. The indices measure in different ways species diversity and species composition in terms of sensitivity. The impact factors in the gradients included organic load, hypoxia, sediment metal contamination, urban effluents and physical disturbance. The indices responded in a similar way, mostly as a threshold response, to the pressure factors irrespective if the factor was organic pollution, metal contamination or other pressures. Usually, the border between *Good* and *Moderate* Ecological Quality Status (EcoQS) according to the Water Framework Directive (WFD), the G/M border, is determined as some deviation from a reference, near-natural, situation. Reference data, however, are difficult to find. An alternative procedure is described here to estimate the G/M border, which does not require near-natural reference data. Threshold values, beyond which faunal structure deterioration commences, were identified from non-linear regressions between indices and impact factors. Index values from the less impacted side of the thresholds were suggested to come from environments of *Good* and *High* EcoQS, and the lower border of these data, estimated by the 5th percentile of data after bootstrapping, should form the G/M border. G/M borders for each coastal area (gradient) were calculated and compared with previous studies.

1. Introduction

1.1 Background for the project

Distribution and composition of marine benthic fauna provide information about environmental conditions as species have different tolerance/sensitivity to stressors. As most macro-benthic animals are stationary and may live for several years, the community structure integrates the environmental conditions over at least some years. Thus, the species-abundance patterns in benthic communities provide valuable information which may be synthesised in indices for environmental quality assessment. The structure of benthic communities are analysed along a gradient of disturbance with the aim to classify the benthic habitat quality. Similarly, temporal changes can be analysed by repeated analysis of the faunal composition of the same areas over time. There are many examples of such assessments along gradients, e.g. of hydrographic conditions (Creutzberg et al., 1984), organic enrichment (Pearson et al., 1982), sewage outlets (Fallesen, 1992) and multiple urban pollution (Mirza and Gray, 1981). In semi-enclosed and horizontally stratified marine water bodies, eutrophication-induced oxygen deficiency can have deleterious consequences for the fauna at the hypoxic end of the gradient (Diaz and Rosenberg, 1995; Diaz and Rosenberg, 2008). Pearson and Rosenberg (1978) have generalised the benthic faunal changes along such gradients of organic enrichment and oxygen deficiency, and listed benthic species that are sensitive or tolerant to various degrees of disturbance. However, there are only few studies of faunal community response to heavy metals (Josefson et al., 2008), and metal pollution often occurs in association with organic enrichment (Swartz et al., 1985).

The structure of the benthic fauna is one of the quality elements used in “The EU Water Framework Directive” (WFD; 2000/60/EC) to assess the benthic Ecological Quality Status (EcoQS) in Europe. Scientists in EU have provided several different indices for the assessment of the benthic status, and most country officials have accepted an index of their own. Most of these indices were discussed in Diaz et al. (2004) and recently in Van Hoey et al. (2007), Pinto et al. (2008), Puente and Diaz (2008), and Borja and Dauer (2008). According to the WFD the benthic status should be classified into one of the following classes: *High*, *Good*, *Moderate*, *Poor* and *Bad*, and all water bodies should have at least a *Good* EcoQS. The border between *Good* and *Moderate* EcoQS (G/M border) marks the point where, according to the WFD, a community goes from an acceptable to an unacceptable state and where action plans should be implemented to improve the ecological status. This difference could correspond to a distinct change in function of the system. Thus, it is

urgent for marine scientists and water managers to assess, with high precision, the border between *Good* and *Moderate* EcoQS, since a *Moderate* or lower status results in costly remediation actions.

1.2. Objective

In the present study we compared how three different indices used by scientists in Denmark, Norway and Sweden correlated to different environmental pressure factors. The pressure factors were organic enrichment, hypoxia, human effluents and heavy metals. We used gradient data, most of them previously published, from seven coastal gradients in Scandinavia, the Skagerrak and the Kattegat, and Greenland. The usual method to determine the G/M border involves some deviation from a reference situation, but valid reference data are difficult to find (e.g. Birk and Hering, 2009). Here we used the results from the gradient data to develop an alternative procedure to determine the G/M border. We focussed on thresholds in the responses to the impacts, points where faunal structure deterioration rates changes. Samples with index values above the threshold are assumed to be in at least *Good* EcoQS. Using the new procedure, the G/M borders in the studied areas were determined for each of the three indices.

2. Material and methods

2.1 Description of the materials used – fauna and environmental factors

2.1.1 Nickel and lead pollution, Kristiansandsfjord

Falconbridge Nikkelverk is a smelter and refinery plant in Kristiansand, South Norway (Fig. 1a). The plant is located close to Hannevika, a bay in the western part of the Kristiansandsfjord. The primary metals refined are nickel, copper and cobalt. Samples of benthic fauna and sediments were taken at 24 stations in the fjord, where 5 stations were sampled twice and the rest one time, during the last three decades. The fjord sediments in the vicinity of the plant are strongly contaminated with heavy metals (Oug et al., 2004; Berge et al., 2007). Here, we analyzed fauna status vs. nickel and lead. Other metals in the sediments (e.g. copper), were significantly correlated with nickel levels, but had fewer data.

Figure 1 a. Map showing investigated area with details on six gradients in caption figures. Stations denoted by dots and location of point sources by asterisk.

Figure 1b. Map showing investigated area with sites (dots) and location of tailings disposal site (asterisk) at Maarmorilik, West Greenland.

2.1.2 Heavy metal contamination gradient, Maarmorilik

The Maarmorilik gradient is situated in two inter-connected fjord arms, Affarlikassa and Quaamarujuk located in the inner part of the Uummanaq fjord system in West Greenland (Fig. 1b). The inner fjord arm has, in particular, received metal contaminated rock wastes and mine tailings from the “Black Angel Lead and Zink mine” (Fig.1b.) in the 1970s and 80s (Johansen et al., 2006). Being crushed rock, the gradient is likely a pure metal gradient with lead, zink and to some extent copper as dominating heavy metals. Tailings were disposed at 40 m depth in the inner fjord arm which had a maximum depth of 70 m. Macrobenthic species abundance and surface sediment metal concentrations were measured at 16 stations, in the tailings area and outside, before, during and 15 years after termination of tailings disposal activities, following a BACI sampling protocol (Johansen et al., 2006; Josefson et al., 2008) The resulting material used here was ca 150 paired faunal and sediment metal (Pb) samples.

2.1.3 Physical disturbance, Jøssingfjord

The Jøssingfjord area, Norway (Fig. 1a) has been used since 1960 for sea disposal of finely ground, inert tailings from a titanium mine. The discharged quantity was about 2 million tonnes/year in the 1980s. The submarine outfall was in 1984 relocated from the shallow Jøssingfjord to the deep basin Dyngadypet outside the fjord entrance. The relocation resulted in increased accumulation within 2–3 km from the new outfall. Tailings

were detectable in the sediments as particles with high titanium content, i.e. 10–15% TiO₂ in tailings compared to a background level of 0.5–1%. Changes in the benthic fauna at varying distances from the old and new outfall sites have been followed, and biological impacts of mine tailings analysed (Olsgard and Hasle, 1993). A sedimentation of 4–5 cm of tailings per year at some locations resulted in changes in faunal composition, while at a rate of 1 mm per year, no impact was observed. This area is the most open and exposed area of the studied gradients.

2.1.4 Urban effluents gradient (PAH's, PCB, copper, ect.) in the Oslofjord

The Oslofjord penetrates inland over a distance of about 100 km from the open Skagerrak to the city of Oslo (Fig. 1a). Approximately 1 million people live in the area. The sediments in Oslo harbour and the innermost part of the fjord are contaminated as a result of industrial activities, boat traffic, urban road traffic, municipal wastewater, and small rivers draining from industrial areas. The fauna in the inner Oslofjord is affected by the urban effluent (Olsgard, 1995; Walday and Olsgard, 2004). Using PCB sediment concentrations as an indicator of urban effluents there is a clear gradient with high concentrations close to harbour of Oslo which are decreasing outwards in the fjord (Fig. 2). Sediment levels of copper, PAHs and other pollutants are high in the innermost part of the fjord and are correlated with PCB.

Figure 2. Distribution of sediment PCB/ concentrations with distance from Oslo harbour in the Oslofjord. Regression line obtained with LOWESS smoothing, tension=0.5.

2.1.5 Urban effluents gradient (nutrients, organic material and heavy metals) in a Danish bight

The Aarhus Bight on the east coast of Jutland (Denmark) (Fig. 1a) is connected to the Kattegat/Belt Sea area and has an average water residence time on the order of 2 weeks. The Bight receives effluents both from the city of Aarhus (ca 500 000 inhabitants) and the surrounding drainage area. The point of discharge is situated close to the harbour of Aarhus (Fig.1a). In addition to nutrients the effluent also contains organic material as well as various contaminants like heavy metals. Benthic macrofauna species and abundance and the sediment variables ignition loss and concentrations of *Clostridium* bacteria (CB) (a marker sewage effluents), were measured at 20 stations in the Aarhus Bight over the time period 1976 to 2005 (Josefson et al., 2006). Water depths at the stations varied between 12 and 17 m. The distances from investigated sites in the bight to the discharge point ranged from ca 1 km to 23 km. In addition, fauna samples were available from a reference area outside the Bight around the island of Samsø in the Belt Sea, some 50 km from the discharge point (Josefson et al., 2006). The total fauna data set with corresponding measurements of sediment variables was around 500 samples each corresponding to a bottom area of 0.1 m². CB data indicated decreasing sewage influence from the discharge point with an inflexion point at ca. 11 km (Fig. 3).

Figure 3. Plot of numbers of *Clostridium* bacteria in the surface sediments versus distance from point source in the Aarhus Bight. Solid line obtained from LOWESS smoothing, tension=0.5. Note the inflexion point at ca 11 km.

2.1.6 Temporal gradients after organic pollution from pulp mill effluents, Saltkällefjord

The benthic communities in Saltkällefjord (inner part of Gullmarfjord, Fig. 1a) were impacted by organic waste from a pulp mill up to 1966, when the factory closed down. The fauna was eliminated at the innermost stations and reduced in the inner half of the fjord. The recovery of the benthic fauna was followed in a number of studies (Rosenberg, 1972, 1973, 1976), and the communities had more or less completed the succession to “normal” conditions in the inner half of the fjord after eight years.

2.1.7 Temporal gradients following oxygen depletion events in a deep fjord on the Swedish west coast

The Gullmarfjord is open towards the west and the Skagerrak with a sill at the entrance of about 40 to 50 m depth and 118 m deep basin (Alsbäck) (Fig. 1a). The deep water in the fjord is generally exchanged once annually, but low oxygen concentrations occur in some years because of limited or no water exchange. The benthic community reduction and recovery at Alsbäck in relation to changing oxygen conditions have been studied by Josefson and Widbom (1988), Nilsson and Rosenberg (2000) and Rosenberg et al. (2002). The available material includes fauna and bottom water oxygen concentration time series over 31 years (1977–2007; Fig. 4g).

2.2 Multi-metric indices

The three Nordic indices are multi-metric and share the same underlying rationale, that the quality of the faunal communities is reflected by both the contribution of sensitive and tolerant species and the species diversity. However, the relative weighting of the diversity and sensitivity components are different, and the indices uses two different classifications systems of the sensitivity of the community, and three different measures of species diversity. Very low numbers (i.e. < 10) of individuals in the samples are in all cases considered as a low quality feature and this is accounted for by using different factors to modify the indices against the number of individuals in the samples. All three indices are calculated on data from a sample size of the bottom of 0.1 m².

2.2.1 The Danish index

The Danish index, DKI described in Borja et al. (2007), uses the AMBI index where species are classified according to sensitivity/tolerance to disturbance (Borja et al., 2000), the Shannon diversity (H; Shannon and Weaver, 1963) and a factor compensating for both low species and den-

sity numbers. The value of AMBI is calculated from the proportions of individuals of sensitive/tolerant species within a sample. The diversity component (H') and the sensitivity component (AMBI) are both normalised to attain a value between 0 and 1, where the diversity is normalised against the highest diversity observed in the sea area. The two components are weighted equally in the calculation of DKI, and the index-value is modified by the factor compensating for low species and density numbers:

$$DKI = \left(\frac{\left(1 - \frac{AMBI}{7}\right) + \left(\frac{H'}{H'_{\max}}\right)}{2} \right) \times \left(\frac{\left(1 - \frac{1}{N}\right) + \left(1 - \frac{1}{S}\right)}{2} \right)$$

where H' the Shannon index with log base 2, and $H'_{\max}$ the reference value which H is normalised against and is the highest value that H reaches in undisturbed conditions. $H'_{\max}$ was set to 5.6 which was the highest value observed in this material, except for Maarmorilik where $H'_{\max}$ was set to 4.0, N the number of individuals and S the number of species.

DKI can attain value between 0 and 1. For very high number of species the value of DKI approaches the mean of the normalised sensitivity and diversity components. If $S=1$ then $0 < DKI < 0.25$ and if $S = 1$ and $N = 1$ then $DKI = 0$

DKI scales linearly with the AMBI-value of the samples for all combinations of S and N

2.2.2 The Norwegian index

The Norwegian index, NQI, also uses the AMBI index (Borja et al., 2000) as a measure of sensitivity and is normalised to attain values between 0 and 1 in the same way as in DKI index. The diversity component is described by a factor, SN (Rygg, 2006) which is normalised to attain values between 0 and 1 and the diversity component is also modified by a factor to compensate for low densities. The normalised sensitivity and diversity components are weighted equally in NQI as described by:

$$NQI = 0.5 * \left(1 - \frac{AMBI}{7}\right) + \left[0.5 * \frac{SN}{2.7} * \frac{N_{tot}}{N_{tot} + 5}\right]$$

where AMBI is the sensitivity component and $SN = \ln(S)/\ln(\ln(N))$ is a diversity index. N is the number of individuals in the sample and S the number of species in the sample. The diversity component is normalised

with a factor of 2.7, which is the maximum value of SN observed in the samples.

NQI can be calculated for values of $N > 1$ and $S < N$ and can attain values between 0 and 1. However, for $S = N$ and $S < 4$ the SN is not a continuous function and calculation of NQI result in outlying index-values. Calculation of the diversity component is independent of the relative dominance (unevenness) of species in the sample.

2.2.3 The Swedish index

In the Swedish index, BQI (Rosenberg et al., 2004; Blomqvist et al., 2006; Anon., 2008), the sensitivity component is based on a classification of sensitive/tolerant species which is used to calculate a weighted average of the sensitivity of a species assemblage. The sensitivity value of 375 species was in this study objectively calculated from species-specific abundance distributions from a large Nordic data set based on 0.1 m² grab data from 800 stations.. Species richness is accounted for in BQI by a factor which scales with logarithm to the number of species and BQI is modified for low densities:

$$BQI = \left[\sum_{i=1}^{S_{classified}} \left(\frac{N_i}{N_{totalclassified}} * Sensitivityvalue_i \right) \right] * \log_{10}(S+1) * \left(\frac{N_{total}}{N_{total} + 5} \right)$$

where $S_{classified}$ denoted the number of species classified. Sensitivity value_i denotes the sensitivity of the ith species which ranges between 1 and 15. Low values indicate a high proportion of tolerant species, and high values indicate a high proportion of sensitive species (Rosenberg et al., 2004). S is the number of species (including not classified) per sample. N_i = the number of individuals of the ith species, $N_{totalclassified}$ = the total number of classified individuals, N_{total} = the total number of individuals per 0.1 m².

BQI may be calculated for all values of S , $S_{classified}$ and N . Theoretically, there is no upper limit for the BQI-value, which increases with the number of species. In practice, S in a sample is never expected to exceed 100. For $N = 1$ BQI ranges between 0.3 and 0.75. For $S = 1$ and high values of N then BQI ranges between 0.3 and 5 depending on the specific sensitivity value. For $S = 0$ then BQI = 0.

2.2.4 Method to determine a G/M border and statistical methods

The G/M border should ideally be determined in relation to some reference situation (Pollard and van de Bund, 2005). In practice, however, reference (i.e. = no or little human influence) situations are difficult to find, and some investigators have instead determined the G/M border from data coming from environments assumed to be in at least Good status (GIG NEA 8 for example, Carletti and Heiskanen draft). At least

Good status was then assessed when fauna data were on the less impacted side of the threshold values of impact (e.g. Birk and Hering, 2009). Here we adopt a somewhat similar approach and screen data from pressure gradients for data of *Good* and *High* status. We have graphically analysed where abrupt changes, or thresholds, occur along different pressure gradients in the benthic community structure estimated by the indices. Index values were regressed against the pressure variable using the smoothing method “Locally weighted least square regression” (LOWESS, Cleveland, 1979), which is an appropriate moving average method when smoothed data points not are equidistant, as often is the case here. For this purpose we used the LOWESS option in the statistical computer package SYSTAT ver. 10. The point on the regression line where rate of change shows a threshold is identified. Index values on the less impacted side of this point are considered to be in a at least “*Good*” ecological status according to the WFD. Regarding these index values as coming from a distribution of values in an environment in at least *Good* status, the G/M border is obtained by taking the 5th percentile of the data on the less impacted side of the threshold after bootstrapping. Bootstrapping (Efron and Tibshirani, 1993) is a powerful method to produce estimates of parameters taken from an unknown probability distribution, which is the case here. The 5th percentile was selected because that is a statistical praxis for one-sided tests. The procedure was as follows: The sample of at least *Good* data was sampled randomly with replacement with the same number as the sample size. This was repeated 9999 times and finally the median 5th percentile and the 95% confidence limits around the median were estimated.

To assess if index values on the good side of the threshold points were different from values on the worse side of the thresholds, data were tested for difference with Kruskal-Wallis ANOVA with two groups.

3. Results

3.1 Identification of pressure thresholds

Relations between the three indices and pressures in the different gradients are shown in Figure 4 analysed with the smoothing method LOWESS.

Overall the figures showed distinct change of all three indices in each of the seven different gradients. In the gradients of metal contaminated sediments outside the Kristiansand Nickel smelter, the three indices all decreased markedly in relation to sediment concentrations of both nickel (Fig. 4a) and lead (the latter not shown in figure because patterns were similar to nickel). Inflexion points in the LOWESS regression lines occurred at Ni concentrations between 500 and 1500 mg/kg depending on index. For lead the threshold values were the same for all indices at around 200 mg/kg, and thus similar to the observed threshold in the Greenland lead gradient.

Figure 4 a. Plots of index values against sediment nickel concentrations in the Kristiansand gradient analysed with LOW-ESS smoothing non linear regression (solid curves, tension=0.5). Threshold value of pressure factor indicated by vertical dashed line, and horizontal dashed lines denote 95% confidence interval around the median 5th percentile (G/M border) of bootstrapped data on the less impacted side of the threshold.

In the sediment lead gradient at the Lead and Zink mine at Maarmorilik, West Greenland all three indices showed dramatic declines in response to increasing lead concentrations (Fig. 4b). Similarly, the lead gradient in the Kristianssand Fjord (Southern Norway) showed inflexion points in the LOWESS curves around lead concentrations of 200 mg/kg.

Figure 4 b. Plots of index values against sediment lead concentrations in the Maarmorilik gradient analysed with LOWESS smoothing non-linear regression (solid curves, tension=0.5). Threshold value of pressure factor indicated by vertical dashed line, and horizontal dashed lines denote 95% confidence interval around the median 5th percentile (G/M border) of bootstrapped data on the less impacted side of the threshold.

In the spatial gradient of tailing deposition in the Jössingfjord area all three indices decreased in relation sediment concentration of TiO_2 which reflected the amount of tailings deposited (Fig. 4c). The main significance of its presence was considered as indicator of physical disturbance by tailing deposition. Although the changes in the indices along the gradient were small, there seemed to be a change in rates of decrease in the concentration interval 3.9–5.5% of sediment TiO_2 .

Figure 4 c. Plots of index values against sediment TiO_2 in the Jössingfjord analysed with LOWESS smoothing non linear regression (solid curves, tension=0.5. Threshold value of pressure factor indicated by vertical dashed line, and horizontal dashed lines denote 95% confidence interval around the median 5th percentile (G/M border) of bootstrapped data on the less impacted side of the threshold.

In the Oslofjord there was a spatial gradient in urban/industrial effluents with inputs of organics, heavy metals as well as polychlorinated hydrocarbons, PCB (Olsgard, 1995; Walday and Olsgard, 2004) with the main point sources located in the harbour area of Oslo (Fig. 2). Plotting the three indices against distance from the point source showed a clear increase of the values with increasing distance from the harbour (Fig. 4d). Inflection points in the LOWESS curves, where the index values levelled off on a high level occurred around 20km from the harbour (Fig. 4d).

Figure 4 d. Plots of index values against distance from the harbour in the Oslofjord impact gradient analysed with LOW-ESS smoothing non linear regression (solid curves, tension=0.5). Threshold value of pressure factor indicated by vertical dashed line, and horizontal dashed lines denote 95% confidence interval around the median 5th percentile (G/M border) of bootstrapped data on the less impacted side of the threshold.

In the other urban/industrial effluent gradient, the Aarhus Bight in Denmark, there was a clear change in sewage influence, measured by *Clostridium* bacteria concentrations in the sediment (Fig. 3), with increasing distance from sewage point source (Fig. 4e). All three indices showed a consistent pattern; an increase and subsequent levelling off, with increasing distance from the sewage point source. The inflexion points occurred around 10 km from the harbour. Unlike the Oslo gradient, the water depth was fairly constant along the gradient, (15–17m) and mixing with water masses in the open part of the Belt Sea is probably more intensive due to the short residence time in the Aarhus Bight.

Figure 4 e. Plots of index values against distance from point source in the Aarhus Bight gradient analysed with LOWESS smoothing non linear regression (solid curves, tension=0.5). Threshold value of pressure factor indicated by vertical dashed line, and horizontal dashed lines denote 95% confidence interval around the median 5th percentile (G/M border) of bootstrapped data on the less impacted side of the threshold.

In the Saltkällefjord, where the pressure factor was organic material from pulp mill effluents and the gradient is shown in the temporal dimension, the index values started at a very low level in the 1960s concomitant with the closure of the mill (Fig. 4f). At this point nearly azoic bottoms prevailed in the area close to the discharge. A recovery process continued until the mid 1970s when the indices stabilised and thereafter remained at a more constant level. Here the inflexion point, demarking the phase in the recovery process where the community begin to stabilise, was set to year 1977 for all three indices.

Figure 4 f. Plots of index values against time in the Saltkällefjord analysed with LOWESS smoothing non-linear regression (solid curves, tension=0.5). hreshold value of pressure factor indicated by vertical dashed line, and horizontal dashed lines denote 95% confidence interval around the median 5th percentile (G/M border) of bootstrapped data on the less impacted side of the threshold.

In the Gullmarfjord deep basin (Figure 4g,h and i), repeated hypoxia with varying time intervals has precluded the straightforward approach used in the other gradients. Here we used two approaches to screen for at least *Good* data: 1. Selection of data from time periods with stable high index levels without occurrence of low oxygen values in the bottom water i.e 1977–80 and 1999–2007 (Fig. 4h).

Figure 4 g. Long-term development of the three indices, BQI, NQI and DKI in the Gullmarfjord Basin. Superimposed is the development of oxygen concentrations in the near-bottom water.

Figure 4 h. Plots of index values against time in the Gullmarfjord basin analysed with LOWESS smoothing non linear regression (solid curves, tension=0.2). Data points outside the vertical dashed lines were assumed to come from at least Good status conditions. Horizontal dashed lines denote 95% confidence interval around the median 5th percentile (G/M border) of bootstrapped values of these data.

2. Harmonisation of all index data against time elapsed from severe hypoxic conditions resulting in defaunation and thereby pooling the evolution of the recovery phases following each of the severe hypoxic events (Fig. 4i). All major events of defaunation followed after oxygen depletion events with oxygen concentrations below 0.5 mg/l over some weeks. The effect of the oxygen depletion event on the fauna community depends on the duration and due to the sampling frequency it was not always possible to determine the exact duration of the hypoxia. Therefore index values were plotted against elapsed time since oxygen was below 0.5 mg/l and an effect was observed in the fauna community during that particular event. This resulted in pooling of the succession schemes of the three recovery events. In the latter approach LOWESS smoothing showed an inflexion point of the recovery phases at about 500 days ~ 2 years after the hypoxia event. In both approaches at least *Good* data were selected from the less impacted side of thresholds obtained with LOWESS smoothing (Figure 4h,i).

Figure 4 i. Plots of index values against time elapsed from defaunation due to hypoxia in the Gullmarfjord basin analysed with LOWESS smoothing non linear regression (solid curves, tension=0.5). Threshold value of pressure factor indicated by vertical dashed line, and horizontal dashed lines denote 95% confidence interval around the median 5th percentile (G/M border) of bootstrapped data on the less impacted side of the threshold.

To assess if index values on the less impacted side of the threshold points were different from values on the more impacted side of the thresholds, data were tested for statistical difference (Table 1). In all instances, both for indices and gradients, P was < 0.001 showing significant difference. The single exception was for the gradients in Kristiansand, with only some P values < 0.01 . This shows that the data from at least *Good* status were significantly higher than data classified as worse status (*Moderate*, *Poor* and *Bad*).

Table 1 Statistical comparison of index values of at least *Good EcoQS* with values at worse status delimited by threshold values of pressure variables using Kruskal Wallis ANOVA. Median values for the two groups of data are shown.

Gradient/index	Median good	Median worse	n good	N worse	MWU	P
Kristiansand Ni / BQI	14.45	8.99	21	8	141	0.005
Kristiansand Ni / NQI	0.64	0.43	19	10	179	0.000
Kristiansand Ni / DKI	0.62	0.30	18	11	188	0.000
Kristiansand Pb / BQI	14.79	9.58	13	8	84	0.020
Kristiansand Pb / NQI	0.62	0.42	13	8	93	0.003
Kristiansand Pb / DKI	0.62	0.27	13	8	93	0.003
Maarmorilik Pb / BQI	9.37	4.17	94	55	4193	0.000
Maarmorilik Pb / NQI	0.64	0.36	101	36	3405	0.000
Maarmorilik Pb / DKI	0.68	0.27	72	77	4700	0.000
Jøssingfjord / BQI	17.19	15.12	11	24	228	0.001
Jøssingfjord / NQI	0.78	0.58	10	25	222	0.000
Jøssingfjord / DKI	0.83	0.47	8	27	201	0.000
Oslofjord / BQI	15.30	6.88	14	67	899	0.000
Oslofjord / NQI	0.66	0.53	19	48	797	0.000
Oslofjord / DKI	0.65	0.39	17	64	965	0.000
Aarhus Bight / BQI	8.91	7.02	169	317	42919	0.000
Aarhus Bight / NQI	0.65	0.59	169	311	41942	0.000
Aarhus Bight / DKI	0.61	0.51	151	335	40727	0.000
Saltkällefjord / BQI	13.57	6.19	128	234	28732	0.000
Saltkällefjord / NQI	0.72	0.55	128	222	26453	0.000
Saltkällefjord / DKI	0.70	0.46	128	234	27282	0.000
Gullmarfjord I / BQI	11.58	8.10	57	132	6084	0.000
Gullmarfjord I / NQI	0.65	0.54	44	125	4848	0.000
Gullmarfjord I / DKI	0.60	0.41	41	148	5298	0.000
Gullmarfjord II / BQI	11.40	3.29	107	82	8115	0.000
Gullmarfjord II / NQI	0.58	0.39	102	67	5710	0.000
Gullmarfjord II / DKI	0.54	0.23	103	86	7745	0.000

MWU = Mann Whitney U-statistic, n = number of values, P = likelihood of a true 0-hypothesis (no difference).

3.2 Comparison of indices

The three indices showed a high degree of covariation as they responded to the gradient of the various pressure factors in a very similar way (Figure 4a-i). Regressions between them (Fig. 5), although positive and highly significant, indicated differences between them. NQI and DKI were more similar than either of them compared to the BQI index.

Figure 5. Plots of the three indices against each other with linear regression lines based on the total material (n~1400).

3.3. Estimating the G/M border

Based on the threshold values identified from the inflexion points, the index values in the *Good* end of the gradients were regarded as representing at least *Good* or *High* status. The lower border of these selected data should represent the *Good-Moderate* border according to the WFD. The estimated G/M borders, 5th percentiles of bootstrapped data, are shown in Table 2 and Figure 4 and 6 for each index and gradient. Apart from the gradient in physical disturbance in the open-coast area outside Jøssingfjord, which showed elevated values for all indices, most estimates of the G/M borders were similar between the gradients (Fig 6).

Table 2. Threshold limits of pressure factors and 5th percentile estimates of the Good/Moderate border based on bootstrapped data from the less impacted side of the threshold.

Gradient	Pressure proxy	Threshold value	Median G/M _{5perc}	Lower 95% CL	Upper 95% CL	Nr of values
BQI						
Kristiansand	Sed Pb	202	10.57	9.96	13.18	13
Kristiansand	Sed Ni	1462	9.96	8.35	10.98	21
Maarmorilik	Sed Pb	227	6.49	4.56	7.44	94
Jøssingfjord	Sed TiO ₂	5.0	15.53	15.16	16.90	11
Oslofjord	Distance m	21365	13.58	13.27	14.51	14
Aarhus Bight	Distance m	10290	5.96	5.27	6.49	169
Saltkällefjord	Year	1977	10.52	9.99	10.85	128
Gullmarfjord I			8.68	7.99	9.29	57
Gullmarfjord II	Days	489	8.26	6.96	8.99	107
NQI						
Kristiansand	Sed Pb	202	0.48	0.47	0.57	13
Kristiansand	Sed Ni	618	0.48	0.47	0.58	19
Maarmorilik	Sed Pb	247	0.41	0.40	0.45	101
Jøssingfjord	Sed TiO ₂	4.4	0.62	0.59	0.78	10
Oslofjord	Distance m	18016	0.57	0.56	0.62	19
Aarhus Bight	Distancem	10290	0.55	0.54	0.56	169
Saltkällefjord	Year	1977	0.61	0.57	0.62	128
Gullmarfjord I			0.55	0.52	0.60	44
Gullmarfjord II	Days	517	0.46	0.41	0.49	102
DKI						
Kristiansand	Sed Pb	202	0.36	0.34	0.48	13
Kristiansand	Sed Ni	524	0.37	0.34	0.53	18
Maarmorilik	Sed Pb	177	0.50	0.28	0.57	72
Jøssingfjord	Sed TiO ₂	2.9	0.77	0.76	0.83	8
Oslofjord	Distance m	19422	0.46	0.44	0.58	17
Aarhus Bight	Distance m	11515	0.46	0.45	0.50	151
Saltkällefjord	Year	1977	0.53	0.46	0.54	128
Gullmarfjord I			0.47	0.46	0.52	41
Gullmarfjord II	Days	517	0.36	0.25	0.43	103

Figure 6. Estimates of Good-Moderate border values for the three Nordic indices in seven pressure gradients. Values are estimated 5th percentiles of at least Good data and Higher status.

4. Discussion

Since the end of the last century several marine scientists have presented different indices for the assessment of benthic habitat quality in European waters. Here we present comparative analyses of three indices from Denmark, Norway and Sweden applied on materials from coastal areas affected by various degrees and types of disturbance. The disturbances include combinations of organic enrichment, hypoxia, heavy metal contamination and physical disturbance by tailings deposition. Despite difference in impact factors, the response by the benthic fauna structure, measured by the indices, shows high similarities. Overall the values of the indices decrease with increasing values of the impact factors. Furthermore, it appears that the response of indices along the various pressure gradients is typically non-linear. At some threshold value of the impact factor there is often a marked change in change rates of the indices, indicating an increased deterioration of the benthic fauna structure. When the pressure exceeds the resistance of the community, for instance considerable species loss will occur. Such threshold responses of benthic macrofauna has previously been described in relation to hypoxia (Diaz and Rosenberg, 1995; Levin and Gage, 1998), organic enrichment in general (Hyland et al., 2005), and sediment metal contamination (Burd, 2002; Josefson et al., 2008).

4.1. Comparison of the three indices

The multi-metric indices include different aspects of the benthic community. One basic element in the Norwegian and Danish indices, is the classification of species according to their sensitivity or degree of opportunism in relation to disturbance, the AMBI index. The AMBI classification method, based on the Pearson and Rosenberg (1978) classification in relation to organic pollution, has proven useful in describing also other impacts (Borja et al., 2003; Muxika et al., 2005). The Swedish index uses a different classification system of the species sensitivity (Rosenberg et al. 2004). The other basic element in the indices used is some measure of species diversity, a measure that has a quality value in itself. Diversity is quantified in terms of species richness in the Swedish index, BQI. SN is used in the Norwegian index and Shannon H in the Danish index. These differences are likely reflected by the variation in the regressions between indices where for instance the smaller variation between NQI and DKI probably is because both indices include the same AMBI index (Fig. 5). The differences between AMBI and the Swedish sensitivity component

are illustrated by Figure 7, where the scatter around the overall positive relations is considerable.

Figure 7. Plots of the two sensitivity factors used in the indices for each gradient. Gullmarfjord (a), Saltkällefjord (b), Kristiansand (c), Oslofjord (d), Jøssingfjord(e), Aarhus Bight (f) and Maarmorilik (g). Grey dots represents grabs where either BQI or AMBI have more than 20% of the individuals not assigned a sensitivity value.

However, despite these differences there was generally an excellent agreement between the three indices in reflecting the response to the pressure factors. This is clearly evident from the close agreement of the estimated threshold values for all gradients (Table 2). The use of different measures of species diversity has advantages and disadvantages and this affects the applicability of the indices to different environments. The DKI and NQI incorporate diversity measures normalised to the highest values of Shannon or SN diversity within that environment. This is an advantage if these reference values are known. However, such reference conditions may rarely exist. The use of SN-diversity requires species richness values of more than 4 species per sample. The use of species numbers in the Swedish method does not depend on either knowledge of a reference of species richness or a minimum number of species. The formulation in this index means that the index value, in theory, has no upper limit.

4.2. Methodological issues

The G/M border should ideally be determined in relation to some reference situation (Pollard and van de Bund, 2005). In this case some reference or high status values were defined and the values between the *High/Good* border and the lowest value divided into four classes where the G/M border was set between class 1 and 2 from the H/G border. In practice, however, reference (= no human influence) situations are difficult to find, and the G/M border is determined either arbitrarily or in relation to a situation as unaffected as possible (the best you can get). The

latter option includes at least two ways. One is to use threshold values of pressure variables to delimit sites/environments that should be in at least *Good* EcoQS and use faunal data from these environments (Birk and Hering, 2009). Another option might be to deduce at least good data from the faunal response along pressure variable gradients. In the present study we explore the latter possibility further by looking for threshold change in different indices along different kinds of pressure/pollution gradients. The basic idea is that the integrity of an ecosystem to some degree can resist pressures or disturbance until a certain point where system changes occur and that this point mark the border between an acceptable status, i.e. Good status or higher and an unacceptable status, i.e. Moderate or worse status, using the WFD terminology. This point should be reflected by a major change in structure and function of the system such as diversity or species composition, and consequently also in the indices. Therefore, we examined regressions between indices and pressure factors for points where change-rates were increased or decreased. Subsequently we regarded the values on the good side of the threshold points as being from an at least Good status situation. Some lower percentile of these data should be an estimate of the border between *Good* and *Moderate* EcoQS according the WFD.

4.3. Assessment of the border between Good and Moderate EcoQS/comparison with previous studies.

Previous studies in the North-East Atlantic area have arrived at index values of DKI between 0.53 and 0.63 (Borja et al., 2007; Carletti et al., draft) as estimates of the G/M border. However, the absolute values of the DKI index is dependent on the value of H_{max} , the maximum Shannon diversity in the actual environment. The value of H_{max} will vary between environments and therefore will depend somewhat on delineation of typology. In Borja et al. (2007) as well as in Carletti and Heiskanen (draft) H_{max} was set to 5.0, whereas for most of the materials in this study H_{max} was 5.6, the highest value found around Skagerrak/Kattegat. The effect on the estimates of the G/M border of DKI in this study of using $H_{max}=5$ instead of $H_{max}=5.6$ is an increase of the index value by 5–10%, depending on the area studied. The span of G/M values for DKI with $H_{max}=5$ is approximately 0.40–0.59 to be compared with 0.58 (Borja et al., 2007) and 0.53 (Carletti et al., draft), which suggests reasonable agreement between the studies. The G/M border for DKI should be somewhere in the interval 0.4–0.65. This is a rather broad interval and it may be questioned how good the definition is of “at least Good status”, because the H_{max} value refers to pristine conditions which is unknown because most all coastal environments are nowadays affected by human activities. However, the Greenland data may be an exception where we can exclude

human impact in the pre-mining period in the Greenlandic fjord when truly pristine conditions were likely to have prevailed.

Previous studies in the North-East Atlantic area have arrived at an index value of NQI of 0.63 as an estimate of the G/M border, when using 2.7 as a maximum value (normalisation factor) for the diversity component SN. This approach is analogous to the use of $H_{\max}$ in the DKI index. A further normalisation factor of 0.78 (assumed reference value) for the whole NQI index is applied to obtain the EQR value (Carletti and Heiskanen, in press).

Rosenberg et al 2004 suggested two different G/M borders, one above and one below the halocline in the Kattegat/Skagerrak area of the North-East Atlantic. By separating the BQI-span into 5 equal classes they arrived at G/M borders of 10.8 above and 12 below the halocline. In a more recent approach based on assessment with the lower confidence limit of the median of all BQI values from a water body Anon (2008) arrived at G/M borders for the same area of 10.3 above and 12 below the halocline. These results agree well with the BQI G/M borders (between 6 and 15.6) suggested for the different gradients in this study.

Thus determination of G/M border from disturbance gradients is one way of deducing undisturbed condition when these are unknown, but this should ideally be done separately for different physical environments (water types according to the WFD). Yet, as seen in Figure 6, the estimates of the G/M borders in the Greenland material are not very different from the estimates in the more southern fjord systems.

5. Conclusions

- A new procedure is described to estimate the border between *Good* and *Moderate* EcoQS in benthic marine coastal environments.
- Benthic macrofaunal data from seven marine coastal areas with gradients of different pollution sources have been analysed with three Scandinavian methods to assess ecological benthic quality according to the WFD.
- The indices/methods responded in a similar way to the pressure factors, irrespective of the pressure being organic pollution or heavy metal contamination. Correlations between the three indices were generally high.
- Changes in the slopes of non-linear regressions between indices and pressure variables were used to identify thresholds where significant deterioration of the faunal communities started.
- Based on these threshold values, suggested to correspond to the point of change from *Good* to *Moderate* status, the *Good-Moderate* border for the indices was estimated.
- The estimated *Good/Moderate* borders showed similar values between different gradients and it is suggested that the border for the Norwegian and the Danish indices, except for the open coast gradient, should be in the interval 0.36 – 0.61 and the Swedish index 6 – 15.6.
- These former results compare well with results from other investigations in the North Sea area.

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Sammenfatning

I dette arbejde sammenligner vi tre Skandinaviske multimetriske indeks for bundfauna struktur for at vurdere marin bentisk økologisk status i syv forskellige forureningsgradienter. Indeksen måler på forskellige måder artsdiversitet og artssammensætning med hensyn til sensitivitet/tolerance ovenfor forstyrrelser. Presfaktorer i forureningsgradienterne omfattede organisk belastning, hypoxi, metalforureninger i sediment, forureninger i almindelighed fra byer, havne og industrier, og ren fysisk forstyrrelse fra tailing deposition. Indeksen svarede på nogenlunde den samme måde ovenfor ændringer af presfaktorer, dette uanset om faktoren var organisk forurening eller metalforurening. Indeksen viste i de forskellige gradienter mer eller mindre tydelige tærskel responser.

Grænsen mellem *God* og *Moderat* økologisk status ifølge Vandrammedirektivet (VRD), G/M grænsen, skal idealt bestemmes som en afvigelse fra en reference situation, d.v.s. fra en situation med minimal menneskelig påvirkning. Reference data er dog svære at finde. En alternativ procedure for at bestemme G/M grænser bliver derfor beskrevet i dette arbejde, en procedure der ikke kræver naturlige reference værdier. Tærskel værdier af presfaktorer, bortom hvilke bundfaunaens struktur begynder at markant forandres eller kollapse, blev identificeret fra ikke-lineare regressioner mellem indeksen og presfaktorerne. Indeks værdier fra før starten af struktur forandringen blev antaget komme fra et miljø i *God* eller *Høj* økologisk tilstand. Den nedre grænse af disse data, estimeret ved 5te percentilen af data efter boot-strapping, blev antaget være et estimat af G/M grænsen. G/M grænser for vert af de syv gradienter blev beregnet og sammenlignet med tidligere undersøgelser.