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Lost in translation? Multi-metric macrobenthos indicators and bottom trawling.

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Abstract

The member states of the European Union use multi-metric macrobenthos indicators to monitor the ecological status of their marine waters in relation to the Water Framework and Marine Strategy Framework Directives. The indicators translate the general descriptors of ecological quality in the directives into a single value of ecological status by combining indices of species diversity, species sensitivity and density. Studies and inter-calibration exercises have shown that the indicators respond to chemical pollution and organic enrichment, but little is known about their response to bottom trawling. We use linear mixed effects models to analyze how bottom trawling intensity affects the indicators used in the Danish (Danish Quality Index, DKI) and Swedish (Benthic Quality Index, BQI) environmental monitoring programs in the Kattegat, the sea area between Sweden and Denmark. Using year and station as random variables and trawling intensity, habitat type, salinity and depth as fixed variables we find a significant negative relationship between the BQI indicator and bottom trawling, while the DKI is related significantly to salinity, but not to trawling intensity. Among the indicator components, the species diversity and sensitivity indices used in the DKI are not significantly linked to trawling, and trawling only affects the BQI when species sensitivities are derived from rarefied samples. Because the number of species recorded per sample (species density) is limited by the number of individuals per sample (density), we expect species density and density to be positively correlated. This correlation was confirmed by a simulation model and by statistical analysis of the bottom samples in which log species density was highly significantly related to log density ($r=0.75$, df=144, $p<0.001$). Without accounting for the effect of density on species density, indicators based on species density will be affected by temporal and spatial variations in density linked e.g. to variable recruitment.
success. When this variation is accounted for by random year and station effects we find log trawling intensity to explain more of the variation in log density than in the indicators currently used to monitor Good Ecological and Environmental Status in the Kattegat. Disregarding random effects and the relationship between density and species density, the impacts of bottom trawling are likely to be lost in the translation of ecological quality into macrobenthos indicators.

Keywords: macrobenthos indicators, bottom trawling, density, species richness, Water Framework Directive, Marine Strategy Framework Directive
1. Introduction

Quantification of the ecological status of marine soft-bottom macrobenthos has become increasingly important in Europe after the implementation of the European Water Framework Directive (WFD; 2000/60/EC) and the European Marine Strategy Framework Directive (MSFD; 2008/56/EC). Both directives contain descriptors of ecological quality and require the status of marine macrobenthos to be assessed and expressed relative to a situation where anthropogenic impacts are either negligible or at a sustainable level (Van Hoey et al. 2010, Borja et al. 2013). However, translating the qualitative descriptors in the directives into quantitative measurable ecological and environmental properties is an ongoing challenge (Van Hoey et al. 2010). So far the translation has relied heavily on the use of ecological quality indicators which have been used to express the current ecological and environmental status in relation to the desired (Rice et al. 2012, Birk et al. 2012). The main purpose of these indicators is to link a specific anthropogenic pressure to a change in ecological quality extracted from a multivariate response (Hiddink et al. 2006, Muntadas et al. 2016, Rijnsdorp et al. 2016). The link between pressure and response is important because the likelihood that managers will act to reduce or remove ecologically adverse pressures depends on the quality and strength of the scientific evidence that action will result in the outcome intended. Without a scientifically well documented causal relation between a particular pressure and ecological status, managers may be less likely to regulate ecologically adverse pressures, in particular if these pressures are generated by human activities that are economically, politically or socially important. Examining how well indicators link pressure to state is therefore important.

The member states of the European Union have been granted considerable flexibility regarding the implementation of the WFD in their national marine waters and, as a result, many have selected their own indicator to quantify the status of their soft-bottom macrobenthic invertebrate fauna (Quintino et al. 2006, Borja & Dauer 2008, Pinto et al. 2009, Josefson et al. 2009, Birk et al. 2012, Borja et al. 2015). Most of these indicators address the normative definitions and terms of the WFD and therefore include estimators of ‘the level of diversity and abundance of invertebrate taxa’ and the proportion of ‘disturbance-sensitive taxa’ (Vincent et al. 2002, Borja et al. 2004). In practice, this means that they combine a diversity index with an expression of the number of individuals or species present in each
sample and a formula reflecting the observed relative occurrence or abundance of disturbance-sensitive macrobenthic taxa. To assess the relative occurrence of disturbance-sensitive taxa the majority of the member states use the AZTI's Marine Biotic Index (AMBI) (Borja et al. 2000), and a few use the sensitivity metric in the Benthic Quality Index (BQI) (Rosenberg et al. 2004), or other metrics. To reflect 'the level of diversity' Shannon's diversity index (H') (Shannon & Weaver 1963) is often used, and to reflect 'abundance of invertebrate taxa', either the number of species recorded or a combination of species recorded and individual density is most often used (Borja et al. 2009). Hence, sensitivity as defined by AMBI or by the BQI, diversity as reflected by H', and some function of the number of species recorded or density are the most common metrics incorporated in the indicators.

Most of the development, testing and inter-calibration of the national macrobenthos indicators have focused on their response to eutrophication, organic enrichment and chemical pollution (Borja et al. 2007, Borja et al. 2015), and comparatively little work has been spent on examining their response to bottom trawling and seabed abrasion. This is problematic because fisheries generated abrasion exerts a significant pressure on soft-bottom macrobenthic communities in many areas (Kaiser et al. 2006, Collie et al. 2016, Eigaard et al. 2016, 2017). Furthermore, the response of the benthic fauna to mechanical abrasion may very well differ from its response to eutrophication, organic enrichment and chemical pollution. According to the widely accepted 'Pearson and Rosenberg model', organic enrichment will initially increase the growth, density and species richness of the macrobenthos (Pearson & Rosenberg 1978, Gray et al. 2002). A further increase in organic enrichment will increase the oxygen uptake of the seabed eventually resulting in hypoxia or anoxia and a decline in species richness due to a reduction in density or disappearance of sensitive species unable to thrive at low oxygen concentrations. In contrast, mobile bottom-contacting fishing gears are known to kill or damage organisms that are sensitive to mechanical abrasion (Kaiser et al. 2006, Clark et al. 2016, Collie et al. 2016, Neumann et al. 2016). A single passage of a bottom trawl will typically kill 20–50% of the benthic invertebrates in the path of the gear (Collie et al. 2016), but the response is variable and depends on the type of habitat (e.g. substrate), the level of natural disturbance (e.g. hydrographic regime), the species composition of the benthic community, and the footprint of the gear in use (Kaiser et al. 2006, van Denderen et al. 2014, 2015, Eigaard et al. 2016, 2017). Where the longer term response of soft-bottom marine
Macrobenthos to organic enrichment is expected to be a uni- or bi-modal change in benthos biomass, density and species richness, the response to an increase in bottom trawling seems more likely to be a monotonic decline in the biomass and density of sensitive organisms that are sampled by bottom corers and grabs (Queirós et al. 2006, Hinz et al. 2009).

There is, however, a fundamental, but frequently neglected problem that can compromise the assessment of biodiversity with bottom corers and grabs. A single sample represents a fixed sampling area and provides an estimate of species density (the number of species per sampling area), and not species richness (the total number of species present in the habitat sampled). Estimates of species density are often highly correlated with the number of individuals recorded in the samples. This correlation is known to complicate analyses of changes in species density (Gotelli & Collwell 2010, Chase & Knight 2013). For instance, if a sample only contains ten individuals, no more than ten species can be identified, irrespective of the total number of species that are actually present in the habitat sampled. Hence, when density changes at a particular location due to e.g. natural fluctuations in recruitment success or increased mortality caused by bottom trawling, the number of individuals contained in each sample will change, and so will the number of species recorded. A change in the number of species recorded can thus be produced both by a change in the number of species occurring at the location and by a change in the density of individuals affecting how likely it is that the species are represented in the samples. Most macrobenthic indicators use species density to quantify ecological quality and may therefore respond to changes in individual density and distribution as well as to the number of species present.

The purpose of this study is therefore twofold: To investigate the response of the current macrobenthos indicators to bottom trawling; and to examine how the link between species density and individual density may affect the indicators. To this end we analyze a dataset from the Danish macrobenthos monitoring program in the Kattegat between Denmark and Sweden. We focus on the response of the multi-metric DKI and BQI indicators used to monitor macrobenthos quality by the two countries in relation to the Water Framework Directive. Both indicators contain similar elements as the majority of macrobenthos indicators used by other EU member states. Using mixed effects models and estimates of trawling intensity around the benthos sampling stations we investigate how the indicators and their components respond to trawling intensity using salinity, habitat type, and depth as co-
variates and station and year as random effects. Finally, we discuss how to evaluate the ecological status of macrobenthic communities in relation to bottom trawling and other anthropogenic pressures.

2. Material and Methods

2.1 Study area

The Kattegat is situated between Sweden and Denmark and has a total area of ~22000 km² (Figure 1). Most of the western part is relatively shallow and sandy with depths between 10 and 20 m, but the northern and eastern parts comprise a complex postglacial seascape with deep muddy canyons down to 150 m in between shallower mounts of mixed sediments and reefs formed by leaking gases (Al’Hamdani et al. 2007). The Kattegat connects the saline North Sea (salinity >30 ppm) with the more brackish Baltic Sea (<20 ppm) and exhibits a strong vertical stratification as well as a horizontal salinity gradient where salinity below the halocline declines from 34 ppm in the north to 28 ppm in the south. An intensive bottom trawl fishery for Norway lobster (Nephrops norvegicus) impacts the deeper (≥16 m) soft-bottom macrobenthic communities (Pommer et al. 2016). In the more shallow sandy areas, a now much reduced bottom trawl fishery for plaice (Pleuronectes platessa) and cod (Gadus morhua) takes place (Svedäng et al. 2010, Cardinale et al. 2010). The Kattegat has been subject to eutrophication and suffered from hypoxic and anoxic events in the 1980's, but since then the amount of nutrients from land has been reduced and the frequency of hypoxic events has declined (Riemann et al. 2016).

2.2 Benthos samples

Benthos was sampled annually on 22 fixed stations using a Haps corer covering an area of 0.0143 m² (Kanneworff & Nicolaisen 1973). At each station five replicate Haps samples were collected in April or May in the years 2005-2008, 2010, 2011 and 2013 (Figure 1). Each Haps sample was carefully flushed through a 1 mm mesh sieve to extract the animals, which were preserved in a 96 % ethanol solution (Josefson and Hansen 2014). In the laboratory, all individuals were sorted and identified to the lowest possible taxon, preferably to the species level, and the number of individuals of each species or taxon was counted. To reduce the
variance the five Haps samples from each station were combined prior to the calculation of
the DKI and BQI indices. At each station estimates of the average near bottom salinity, depth
and sediment type at EUNIS (European Nature Information System) habitat level 3 were
available.

2.3 Trawling intensity

The area swept by trawling was estimated within a circle with a radius of 2 km centered at
each benthos station. Recruitment of most benthic species in the area takes place from early
spring until late autumn and many of the organisms present in the samples in late April or
early May will be surviving recruits from the previous year. At each station trawling intensity
was therefore cumulated over the period from May in the preceding year to April in the year
where the bottom samples had been collected. The area swept was estimated by combining
data from the Danish Vessel Monitoring System (VMS) with logbook data and estimates of the
towing speed and dimensions of the trawl gears that had been used. Before 2012, the VMS
was only mandatory for vessels longer than 15 m, but although some smaller bottom trawlers
fish in the Kattegat, vessels $\geq 15$ m constitute by far the largest part of the bottom trawlers
(Danish AgriFish Agency 2016). Vessel speed was used to separate actively fishing vessels
from steaming and idle vessels. To calculate the footprint for each logbook-registered fishing
trip, we used the relationships between gear dimensions and vessel size (e.g. trawl door
spread and vessel engine power (kW)) from Eigaard et al. (2016) for different gear types,
vessel groups and target species. Combined with vessel tracks based on the VMS positions and
the interpolation method of Hintzen et al. (2010) these data were used to calculate trawling
intensity, defined as the ratio of the annual area swept to the size of the circular area
surrounding each station. The average trawling intensities ranged from 0 times per year to 73
times per year at the stations in the central part of Kattegat (Figure 1).

2.4 Macrobenthos Quality Indicators

The current version of the Danish Quality Indicator (DKI) is described in Henriksen et al.
(2014). It combines the AMBI index of Borja (2000), where species or taxa are classified
according to their sensitivity to organic enrichment and pollution, the number of individuals
N, and Shannons diversity index $H'$, calculated using log$_2$. The AMBI and Shannon indices
were both standardized by means of empirical salinity regressions derived from another set of reference samples (Table 1).

The Benthic Quality Index (BQI) was calculated from the formula presented in Leonardsson et al. (2009), who also presents sensitivity values for a range of species estimated from a large collection of reference samples in the Kattegat and Skagerrak. The assumption behind the BQI index is that sensitive species can be characterized by occurring in samples with a high number of species, while tolerant species are found in samples with a low number of species (Rosenberg et al. 2004). After using the formula of Hurlbert (1971) to calculate the expected number of species to be found in rarefied reference samples of 50 individuals, the sensitivity of species \( i \), \( \text{Sens}_{E,i} \), is estimated as the lower 5% percentile of the expected number of species found in all the reference samples in which species \( i \) is present. A high sensitivity value thus signifies that a species would tend to occur in areas of high species density.

Because the sensitivity of a species in the BQI index is determined from its relative occurrence in the reference samples, sensitivity will depend on the number and mixture of reference samples available from disturbed and undisturbed environments. When Leonardsson et al. (2015) updated the sensitivities used in Leonardsson et al. (2009) they included reference samples dominated by high numbers of juveniles of one or two species. The high numbers of juveniles in these samples were found to decrease the sensitivity estimates of the other species represented in the samples. Leonardsson et al. (2015) therefore decided to abandon rarefaction in the sensitivity calculation, and changed the base for calculating the sensitivities from the rarefied number of species to the observed number of species. The new species sensitivity, \( \text{Sens}_{O,i} \), was defined as the 5th percentile of the observed number of species each individual of species \( i \) encountered in the reference samples where \( i \) was present (Leonardsson et al. 2015). To examine the effect of this approach we also estimated the BQI indicator \( BQI_{O,j} \) for each sample based on \( \text{Sens}_{O,i} \), the weighted sum of the revised species sensitivities, \( \text{Sens}_{O,i} \), provided by Leonardsson et al. (2015).

2.5 Statistical modeling
All variables were initially examined by pairwise plots and Pearson correlations to reveal the shape of potential relationships and the patterns of interaction. An analysis of covariance was then used to assess the relative importance of the variables used to calculate the DKI and BQI indicators and the Shannon index, while log linear mixed effects models were used to analyze the relationships between the indicators and environmental variables. The log linear mixed effects models used log trawling intensity, EUNIS habitat, log depth and log salinity as fixed effects while station and year were assumed to be random effects considered to reflect random differences in community attributes between stations as well as random inter-annual changes in benthic recruitment success. The analyses of the mixed models were performed in R (R Core Team 2015) using the lme4 and lmerTest packages (Bates et al. 2015). Residual plots and Q-Q plots were inspected for deviations from homoscedasticity and normality. If necessary, variables were log(x+1) rather than log transformed to include zero observations. Parameter estimates were obtained using restricted maximum likelihood and significant variables were identified using backwards elimination of model terms. Alternative model versions were compared using maximum likelihood and Bonferroni adjusted likelihood ratio tests. Only natural logarithms were used.

The initial correlation analysis revealed a linear and highly significant relationship between log density and log species density (r=0.75, df=144, P<0.001, Figure 3) indicating that it was necessary to standardize species density to account for differences in the number of individuals recorded per sample across stations and years.

When only a small fraction of the individuals in a habitat or community is sampled, the number of species recorded provides an underestimate of total species richness which is biased against rare species. This problem was first described for marine benthos by Sanders (1968) and is often solved by individual-based rarefaction where the number of species observed is standardized to the expected number of species observed in a sample containing the same number of individuals, n, as the smallest sample in the group of samples being compared. Rarefying a sample from N to n individuals can mathematically be solved as a combinatorial problem providing an analytical formula for estimating the expected number of species in a random sample of n individuals drawn from a larger N individual sample (Hurlbert 1971, Heck et al. 1975). This, however, assumes that the spatial distribution of the individuals in the environment is random. If the spatial distribution is patchy, rarefaction of
large samples tends to overestimate the number of species in small samples (Gotelli & Colwell 2011). Previous investigations have found that the distribution of benthos in the Kattegat is patchy (Josefson 2016). Furthermore, our samples contained between 15 and 547 individuals necessitating us to rarefy all samples to 15 individuals. Instead of using rarefaction to standardize the number of species prior to our statistical analysis we therefore decided to include a species accumulation curve directly in the statistical model.

A species accumulation curve describes the curvilinear relationship between the number of individuals sampled and the number of species identified. Following the approach of Azovsky (2011) we used a power function to describe this relationship and linearized it by using log species density and log density in the analysis. This allowed us to use the linear mixed effects model to investigate whether trawling intensity significantly affected the relationship. Note that a species accumulation curve generally is used to express the relationship between the number of species identified and the cumulative number of samples or individuals examined from a particular habitat or community (see Gotelli & Colwell 2001). Here we assume that a single species accumulation curve can be used to model samples from different locations and environmental conditions when environmental covariates and random effects of year and stations are simultaneously accounted for.

To examine how removal of species and individuals due e.g. to trawling might affect the shape of the accumulation curve, we also developed a simple stochastic benthic community model where a lognormally distributed species density distribution was randomly generated for 100 species, using the same mean and standard deviation as found in the samples (mean=1.3, stdev=1.3). We then sequentially removed the most abundant, the least abundant, or a randomly selected species from the community and fitted species accumulation curves to the results. We also investigated the effect of removing different proportions of the individuals from all of the species.

3. Results

The pairwise plots and Pearson correlations reveal important and significant linkages between the independent and dependent variables. The most significant interactions are
presented in Figures 2 to 4 and the full correlation matrix is shown in the supplementary material (Figure S1). Log $N_j$, log $S_j$, $DKI_j$, $BQI_{E,j}$, and $Sens_{E,j}$ were all negatively related to log trawling intensity, while $Sens_{O,j}$ was significantly positively correlated to log trawling intensity, and the $Shannon_j$, $AMBI_j$, and $BQI_{O,j}$ indices did not change significantly with log trawling intensity (Figure 2). Log species density, log $S_j$, and log density, log $N_j$, were highly significantly positively correlated (Figure 3). Furthermore, trawling intensity and $BQI_O$ were positively related to salinity, while both $DKI$ and $BQI_E$ declined with salinity (Figure 4).

The analysis of covariance showed that 54% of the observed across sample variation in the $DKI$ indicator was attributed to variation in the Shannon index, and 37% was attributed to salinity (Table 2). The Shannon index was dominated by changes in $S$ which explained 69% of the variation of the index. The variation of the $BQI_E$ was significantly related to changes in both log $S_j$ and in the Sensitivity index, $Sens_E$, which explained 68% and 27% of the variation in the indicator, respectively. The same two indices affected the $BQI_O$ where they explained 56% and 40% of the variation, respectively.

The linear mixed effects model confirmed that log $N$ was highly significantly negatively related to log trawling intensity (Table 3). This effect was not just caused by a few stations. Removing the random station effect from the model and estimating a separate slope for each station revealed that log $N$ declined with log trawling intensity on 18 of the 22 stations, and that the decline was statistically significant for 11 stations. Log $S$ was found to be linearly and highly significantly positively related to log $N$, but not to log trawling intensity, nor to any of the other environmental variables. There was furthermore no significant interaction between the slope of this relationship and log trawling intensity. The linear mixed-effects model showed that the $DKI$ indicator responded significantly to salinity, while the $AMBI$ and Shannon indices neither responded significantly to log trawling intensity nor to any of the other environmental variables. The $BQI_E$ and its associated sensitivity index both responded significantly to log trawling intensity, but not to log salinity or log depth (Table 3). This relationship disappeared when species sensitivities were based on the observed number of species. $BQI_O$ did not respond significantly to any of the explanatory variables, while $Sens_O$ was highly significantly related to salinity. Table 4 provides a full account of the model.
reduction including AIC-values (Akaike's Information Criteria; AIC) and significance of model comparisons.

Using the stochastic benthic simulation model revealed that linear relationships between log density and log species density could indeed be generated by removing either species or individuals from a simulated assemblage. The linear relationships had slopes between 0.7 and 1.5 depending on whether species were removed at random or according to ranked abundance (Figure 5). Removing a fixed proportion of the individuals from each species generated a slope in the species abundance regression of 0.50, not significantly different from the slope of 0.53 estimated from the data (Table 3).

4. Discussion

4.1 Indicator performance

The DKI indicator was found to be significantly negatively related to salinity, but not to trawling. This response was puzzling, because neither the AMBI nor the Shannon index responded significantly to any of the fixed variables included in the mixed effects model. The significant salinity response of the DKI may, however, have been introduced by the salinity standardization which was done without considering the potential effects of differences in trawling intensity, eutrophication, and frequency of hypoxia events that could have influenced density and species density at each of the reference sampling stations. Using salinity as the sole explanatory variable in the standardization may produce a salinity corrected indicator where salinity unintendedly provides the best explanation for the changes observed. In the Kattegat, most of the *Nephrops* trawl fishery takes place below the halocline in the northern deeper parts, where salinity is higher than in the shallower southern part, and salinity and trawling intensity is therefore positively correlated ($r=0.52$, df=146, $P<2.72\times10^{-11}$, Figure 4). The standardization may thus inadvertently have removed the effect of bottom trawling and explained it as an effect of salinity. Adding a log trawling intensity term to the reduced DKI model where salinity was the only fixed term did not improve the goodness of fit (ANOVA, $P=0.23$, df=1) although salinity and log trawling intensity are significantly and positively related.
The only macrobenthos indicator that responded significantly to trawling intensity in the linear mixed effects model was the $BQI_E$. The response was negative and highly significant and was caused by a combination of declines in the average species sensitivity and in the number of species recorded per station (Figure 2). The closely related $BQI_O$ indicator did not respond. The main difference between the two indicators is the way that species sensitivities are calculated. The $BQI_E$ uses species sensitivities based on rarefied species density estimates, while the $BQI_O$ uses the observed number of species without rarefaction. Whether or not to rarefy the species density estimates in the sensitivity calculation has previously been subject to some debate. Fleischer et al. (2007) found the BQI, as defined by Rosenberg et al. (2004), to be sensitive to sampling effort and therefore recommended to rarefy all species density estimates used in the formula, a practice subsequently followed e.g. by Fleischer & Zettler (2009), Grémare et al. (2009) and Chuševé et al. (2016). Leonardsson et al. (2009), however, retained the practice of only rarefying the species density estimates in the reference samples used for estimating species sensitivities, but not the number of species recorded at each station ($BQI_E$), while Leonardsson et al. (2015) decided not to rarefy any of the species density estimates ($BQI_O$), because this led to very low sensitivity estimates for species occurring in reference samples dominated by high numbers of juveniles of one or few species. There may be reasons for using the observed number of species to calculate sensitivities during the period when larvae settle and juveniles are abundant, but our results (see Figure 2) show that this can lead to a significant positive relationship between trawling intensity and sensitivity, and therefore decrease the ability of the BQI indicator to monitor the impacts of fisheries induced mortality. Using the revised unrarefied species sensitivity values from Leonardsson et al. (2015), the abundance weighted overall sensitivity and indicator values were no longer significantly related to trawling. We cannot distinguish whether this was due to the inclusion of samples from the settling period, where the effect of local pressures at the seabed such as bottom trawling may not yet have affected species densities, or whether it was caused by using unrarefied reference samples.

The sensitivity, species diversity, and density components of the multi-metric indicators we have analyzed are contained in most of the national quality indicators of marine macrobenthos that are used to define and monitor the ecological status of coastal and marine waters throughout Europe. However, the species diversity and in some cases also the
sensitivity indices depend on comparable estimates of species density across stations and years. Species density influenced the DKI indicator substantially through the Shannon index, and explained more than half the variation in the BQI_E indicator and the Shannon indices. Only the BQI_O indicator was more sensitive to changes in the weighted species sensitivities at each sampling station than to log species density.

We furthermore found log species density to be highly significantly related to log density. If density varies between years due to natural differences in larval recruitment, the indicators are likely to provide a variable background for estimating of how species diversity may respond to anthropogenic pressures acting on the seafloor, such as bottom trawling. Finally, the linear mixed effects model explained 78 % of the variation in the density data, and 72 % of the variation of the BQI_E (Table 4). Based on these results, we thus find the density of benthic invertebrates to be a better indicator of bottom trawling than any of the present indicators used to monitoring the ecological quality of soft-bottom macrobenthos in the Kattegat.

4.2 Methodological implications

It is often forgotten that quantitative sampling devices such as bottom grabs and corers only provide a count of the number of species per surface area sampled and not an un-biased estimate of the total number of species present in the habitat sampled (Gotelli & Colwell 2001). The difficulty arises because the number of individuals caught per sample limits the number of species that can be recorded per sample, generating a causal link between species density and individual density. When small bottom corers, such as the Haps, are used a typical sample may contain between 10 and 100 individuals, while more than 1000 benthic macroinvertebrate species have been recorded in the Kattegat and western Baltic (HELCOM 2012). Clearly only a fraction of these species will be recorded in a single sample. Exactly how many depends on the size of the local species pool, the spatial distribution of the individuals and/or species, and the number of individuals caught.

By simulating the relationship between species density and individual density in bottom samples we confirmed that the exponent of the species accumulation curve was sensitive to whether species were orderly or randomly removed. The slope in double-logarithmic plots of this relationship was steepest when the least abundant species were sequentially removed and shallowest when all species abundances were gradually reduced in the same proportion.
Interestingly, the slope generated by the analysis of the empirical data was not significantly different from the slope generated by simulating a proportional reduction in abundance for all species (Figure 5d).

Log species density and log density were both highly significantly correlated to each other and to trawling intensity, but trawling did not seem to affect log species density above the effect generated by its reduction of log density. When log density was included in the model of log species density, the impact of trawling intensity on log species density was no longer significant. There was also no significant effect of trawling intensity on the slope of the relationship between log density and log species density. This suggests that log species density is negatively affected by trawling simply because trawling reduces the density of individuals. Had trawling affected the most abundant species more than the less abundant the slope of the relationship between log density and log species density would probably have steepened in response to trawling as shown by the simulations. The slope at the base of the rarefaction curve has been shown to be equivalent to Hurlbert’s probability of interspecific encounter, which is a common sample size independent measure of evenness (Olszewski 2004, Chase & Knight 2013). Hence, because a rarefaction curve would correspond to the lower part of the species accumulation curve a constant logged species accumulation slope suggests that evenness is unaffected by fishing.

Although the slope of the log species accumulation curve thus appears to be resilient to trawling, several decades of trawling could nevertheless have led to a gradual change in species composition that would be important to monitor, but difficult to identify with the present indicators. For instance, if changes in trophic interaction and interspecific competition resulted in species replacements, but the overall relationship between density and species density remained the same, indicators neglecting species identity might not respond. However, previous investigations in the Kattegat have not suggested that species replacements are likely to have happened. These investigations found inter-annual changes in benthos abundance and recruitment to affect all species and all investigated locations similarly, and suggested that a common factor could be operating, perhaps linked to the deposition of organic material on the seabed (Josefson 1987, Josefson et al. 1993) or to general climatic oscillations (Tunberg & Nelson 1998). Furthermore, Pommer et al. (2016) found no relationship between bottom trawling intensity and changes in macrobenthos
community composition in the Kattegat. Zettler et al. (2017) investigated a 30 year time-
series of benthos data from the western Baltic and concluded that benthic communities were
influenced by a multitude of environmental variables and did not appear to be tightly
controlled by any single environmental driver even within a restricted spatial area. We
conclude that this calls for including environmental drivers as well as random year and
station effects in the analyses in order to make anthropogenic impacts identifiable on a
background of substantial natural variation.

4.3 Perspectives

A new generation of indicators is now being developed for monitoring macrobenthos status in
relation to bottom trawling and MSFD requirements. Some of these indicators are based on
changes in species or trait compositions (e.g. longevity) (Hiddink et al. 2006, Eigaard et al.
2017), and may suffer from the same sampling problems as the classical species density and
diversity based indicators used to assess Good Ecological Status in relation to the WFD. We
hope to have demonstrated that mixed effects models provide a possibility for dealing with
some of these problems and allow a more precise translation of the qualitative descriptors of
the directives into quantitative measurable goals. Using linear mixed-effects models of density
solves the problem of standardization across different sources of variation by allowing
incorporation of random effects of e.g. space (station) and time (month, year), generated by
station specific differences in environmental conditions and by inter-annual differences in
recruitment success, as well as fixed effects generated by quantified variables such as salinity,
deepth, bottom habitat and trawling intensity. Incorporation of environmental covariates and
random effects allows changes in density to be mechanistically linked to differences in
anthropogenic and natural pressures. Direct effects of fisheries generated mortality on
macrobenthos communities can potentially be separated from indirect effects by examining
how e.g. growth or reproduction is affected by trawling intensity, providing a possibility for
defining limit reference points of relative densities below which offspring production can no
longer secure replacement.

Finally, relative density could prove useful as an indicator of bottom trawling in parallel with
other indicators. The AMBI has been shown to respond consistently to organic enrichment
and pollution (Borja et al. 2015), but has been found to be less responsive to physical
disturbance (Muxika et al. 2005). We found no significant correlation between AMBI and either density (r=0.062, p=0.46, df=144), log density (r=0.10, p=0.22, df=144) or log trawling intensity (r=-0.002, p=0.98, df=144) in the Kattegat data showing that although AMBI might be used as an indicator of chemical pollution, eutrophication and organic enrichment it is unaffected by trawling intensity. Using several uncorrelated indicators, each responding to a specific pressure, might provide the most unequivocal translation of the impacts of anthropogenic pressures to ecosystem status and could help managers prioritize the measures needed to achieve Good Ecological and Environmental Status in relation to the WFD and MSFD targets for soft-bottom macrobenthos communities.

Acknowledgements
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Borja, Á., Marín, S. L., Muxika, I., Pino, L., & Rodríguez, J. G. (2015). Is there a possibility of ranking benthic quality assessment indices to select the most responsive to different human pressures?. *Marine pollution bulletin, 97*(1), 85-94.


Table 1. Formulas used to calculate the DKI and BQI indicators

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Formula</th>
<th>Where:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danish Quality Indicator (DKI)</td>
<td>(DKI_j = \left[\left(1 - \frac{(AmBj - AmBI_{j,\text{min}})}{7} \right)^{H'<em>{j,\text{max}}} \left(1 - \frac{1}{N</em>{j,\text{total}}}\right)\right]^{\frac{1}{2}})</td>
<td>(H'_{j,\text{max}} = 2.117 + 0.086 \times \text{salinity}<em>j) &lt;br&gt;(AMBI</em>{j,\text{min}} = 3.083 - 0.111 \times \text{salinity}_j)</td>
</tr>
<tr>
<td>Benthic Quality Index (BQIₘ)</td>
<td>(BQI_{E,j} = \sum_{i=1}^{S_{j,\text{classified}}} \left(\frac{N_{j,i}}{N_{j,\text{classified}}} \times \text{Sens}<em>{E,i}\right) \log</em>{10} \left(\frac{N_{j,\text{total}}}{N_{j,\text{total}} + 5}\right))</td>
<td>(N_{j,i}) number of individuals that belongs to species (i) in sample (j). &lt;br&gt;(N_{j,\text{total}}) total number of individuals in sample (j). &lt;br&gt;(N_{j,\text{classified}}) total number of individuals of species with known sensitivity value in sample (j). &lt;br&gt;(S_{j,\text{classified}}) number of species with known sensitivity present in sample (j). &lt;br&gt;(\text{Sens}_{E,i}) sensitivity of species (i) calculated from the expected number of species in reference samples rarefied to 50 individuals.</td>
</tr>
<tr>
<td>Benthic Quality Index (BQI₀)</td>
<td>(BQI_{O,j} = \sum_{i=1}^{S_{j,\text{classified}}} \left(\frac{N_{j,i}}{N_{j,\text{classified}}} \times \text{Sens}<em>{O,i}\right) \log</em>{10} \left(\frac{N_{j,\text{total}}}{N_{j,\text{total}} + 5}\right))</td>
<td>(S_{j,\text{classified}}) number of species with known sensitivity present in sample (j). &lt;br&gt;(\text{Sens}<em>{O,i}) sensitivity value of species (i) calculated from the observed number of species in reference samples. &lt;br&gt;(H'</em>{j}) Shannon diversity index of sample (j) calculated using (\log_{2}). &lt;br&gt;(H'_{j,\text{max}}) predicted maximum Shannon diversity in sample (j) given local salinity.</td>
</tr>
</tbody>
</table>

Danish Quality Indicator (DKI) <br>Henriksen (2014) <br>

Benthic Quality Index (BQIₘ) based on sensitivity estimated from the rarefied number of species <br>Leonardsson et al. (2009) <br>

Benthic Quality Index (BQI₀) based on sensitivity estimated from the observed number of species <br>Leonardsson et al. (2015) <br>
Table 2. Analysis of covariance of the DKI, BQI and Shannon indicators.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Variable</th>
<th>Degrees of freedom</th>
<th>Sum of Squares</th>
<th>F-value</th>
<th>P(&gt;F)</th>
<th>% of Total Sum of Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DKI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMBI</td>
<td>1</td>
<td>0.111</td>
<td>2584.0</td>
<td>&lt;2e-16</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>$H'$</td>
<td>1</td>
<td>0.728</td>
<td>16903.5</td>
<td>&lt;2e-16</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>1/N</td>
<td>1</td>
<td>0.006</td>
<td>134.9</td>
<td>&lt;2e-16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>salinity</td>
<td>1</td>
<td>0.493</td>
<td>11474.9</td>
<td>&lt;2e-16</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>141</td>
<td>0.006</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>BQIE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{ne}$</td>
<td>1</td>
<td>112.3</td>
<td>966.289</td>
<td>&lt;2e-16</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>logS</td>
<td>1</td>
<td>287.2</td>
<td>2470.158</td>
<td>&lt;2e-16</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1</td>
<td>6.3</td>
<td>54.304</td>
<td>1.29E-11</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>142</td>
<td>16.5</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><strong>BQIO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{ne}$</td>
<td>1</td>
<td>1045.9</td>
<td>2185.715</td>
<td>&lt;2e-16</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>logS</td>
<td>1</td>
<td>726.2</td>
<td>1592.767</td>
<td>&lt;2e-16</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1</td>
<td>6.9</td>
<td>14.454</td>
<td>2.13E-04</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>142</td>
<td>68.0</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><strong>H'</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1</td>
<td>13.3</td>
<td>116.31</td>
<td>&lt;2e-16</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>1</td>
<td>64.5</td>
<td>565.93</td>
<td>&lt;2e-16</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>143</td>
<td>16.3</td>
<td></td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>
Table 3. Result from fitting a linear mixed effects model with Year and Station as random variables and habitat, log(depth), log(salinity) and log(trawling intensity+1) as fixed independent variables to various response variables. Only the significant parameter estimates are included in the final models and table. Log stands for natural logarithm, standard error is shown in brackets, grey area signifies not investigated. Significance: *:P<0.05; **:P<0.01; ***P<0.001

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Intercept</th>
<th>logN</th>
<th>log (salinity)</th>
<th>log(trawling + 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DKI</td>
<td>2.36(0.37)**</td>
<td></td>
<td>-0.48(0.11)**</td>
<td></td>
</tr>
<tr>
<td>AMBI</td>
<td>1.71(0.06)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H'$</td>
<td>3.44(0.15)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQI_E</td>
<td>11.81(0.59)**</td>
<td></td>
<td>-1.14(0.32)**</td>
<td></td>
</tr>
<tr>
<td>Sens_E</td>
<td>8.71(0.15)**</td>
<td></td>
<td>-0.35(0.11)**</td>
<td></td>
</tr>
<tr>
<td>BQI_O</td>
<td>16.81(0.98)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sens_O</td>
<td>-17.61(6.77)*</td>
<td>9.03(1.98)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>logS</td>
<td>0.67(0.21)**</td>
<td>0.53(0.04)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>logN</td>
<td>4.86(0.17)**</td>
<td></td>
<td>-0.29(0.09)**</td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>R²</td>
<td>AIC</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>logN = logtrawl + habitat + logsalinity + logdepth + e_{station} + e_{year} + e_0</td>
<td>0.78</td>
<td>214.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>logN = logtrawl + logsalinity + logdepth + e_{station} + e_{year} + e_0</td>
<td>0.78</td>
<td>211.8</td>
<td>0.383</td>
<td></td>
</tr>
<tr>
<td>logN = logtrawl + logdepth + e_{station} + e_{year} + e_0</td>
<td>0.78</td>
<td>210.3</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td>logN = logtrawl + e_{station} + e_{year} + e_0</td>
<td>0.77</td>
<td>209.8</td>
<td>0.230</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4. Backwards model reduction by removal of insignificant terms and likelihood ratio tests.** Selected models are shown in bold types. The R² is between predicted and observed values. AIC is Akaike's Information Criteria and P is the probability from a likelihood ratio test that the model explains the data significantly better than the previous model with the additional term. Significance is Bonferroni corrected to account for the number of model comparisons. *:*P<0.05; **:*P<0.01; ***:*P<0.001. Log stands for natural logarithm.
Figure 1. Map of sampling stations.
Figure 2. Linear regressions of $\log N$, $\log S$, $H'$, AMBI, DKI, BQI and Sensitivity versus log trawling intensity. Asterisks show level of significance: *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$.
Figure 3. Linear relationship between log density and log species density.
Figure 4. Log trawling intensity, DKI, BQI_E and BQI_O versus salinity.
Figure 5. Simulated relationship between number of individuals and number of species.

Abundance of 100 species drawn at random from a lognormal distribution with a mean and standard deviation of 1.3. Graphs show species abundance and number of species subject to a) sequential removal of the least abundant species, b) sequential removal of the most abundant species, c) random removal of species, and d) overall percentage reduction in abundance.
Figure S1. Pairs plot of dependent and independent variables with associated Pearson correlation coefficients. *: P<0.05; **: P<0.001; ***: P<0.0001