Application of Signal Detection Theory approach for setting thresholds in benthic quality assessments

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Abstract

The European Marine Strategy Framework Directive requires EU Member States to prepare national strategies and manage their seas to achieve Good Environmental Status (GES) by 2020. There are many multimetric indices proposed as indicators of the ecological quality of the benthic environment. Their functionality and utility are extensively discussed in the literature. Different frameworks are suggested for comparative assessments of indicators with no agreement on a standardized way of selecting the most appropriate one. In the current study, we apply Signal Detection Theory (SDT) to evaluate the specificity and sensitivity of the Benthic Quality Index (BQI), its response to the eutrophication pressure, and its performance under the effects of estuarine water outflow. The BQI showed acceptable response to total nitrogen, total phosphorus and chlorophyll-*a* concentrations in the study area. Based on the results, we suggest using SDT for setting GES thresholds in a standardized way. This aids a robust assessment of the environmental status and supports differentiation between the quality classes.

Keywords – macrozoobenthos, GES, BQI, sensitivity, specificity, Baltic Sea

1. Introduction

The European Marine Strategy Framework Directive (MSFD) requires EU Member States to align national legislative policies and appropriately manage their seas in order to achieve Good Environmental Status (GES) by 2020 (MSDF; European Commission 2008/56/EC). GES is defined as 'clean, healthy and productive seas within their intrinsic conditions, and the sustainable use of the marine environment'. The directive requires application of a set of indicators for environmental status assessment. When GES criteria are not met, the corresponding measures for achieving them must be specified and undertaken.

Obviously, an adequate and efficient management strategy for the improvement of environmental status implies a robust and reliable status assessment. The crucial step here is the selection of appropriate indicators, therefore many research projects specifically address this issue (Ferreira et al., 2011; Rice et al., 2012; ICES, 2013). A few selection criteria have been suggested, including (but not limited to) scientific basis, responsiveness, range of applicability, data availability, practicality, harmonization, accuracy and confidence (Rice and Rochet, 2005; Niemeijer and de Groot, 2008; Elliott, 2011). Several evaluation methods and conceptual frameworks have been discussed to facilitate decision-making (Borja and Dauer, 2008; Kershner et al., 2011; ICES, 2013). The responsiveness of an indicator is often distinguished amongst the selection criteria (Rombouts et al., 2013). Once an indicator has been developed, its performance in terms of sensitivity (response to an existing disturbance), specificity (resistance to the noise or non-targeted disturbances) and the accuracy in relation to the actual response can be evaluated (Murtaugh, 1996).

It is assumed that benthic species and communities reflect natural and anthropogenic changes in marine ecosystems as they are unable to avoid unfavourable conditions, have a long reproductive cycle, accumulate changes over time and occur at various depths (Zettler et al., 2007). A series of multimetric indices have been proposed to supply synoptic information about the state and ecological quality of the benthic environment, e.g. the Benthic Quality Index (BQI; Rosenberg et al., 2004; Leonardsson et al., 2009), the AZTI Marine Biotic Index (AMBI; Borja et al., 2000), the Biotic Index (BENTIX; Simboura and Zenetos, 2002), the Benthic Opportunistic Polychaeta Amphipoda Index (BOPA; Dauvin and Ruellet, 2007) and the Benthic Opportunistic Annelida Amphipods Index (BO2A; Dauvin and Ruellet, 2009). Yet the performance of these indicators is unlikely to be consistent across habitats and ecosystems, since bottom-dwelling organisms are not equally sensitive to different types of anthropogenic and natural disturbances (Buhl-Mortensen et al., 2009), or environmental conditions (Tagliapietra et al., 2009). Many authors agree that eutrophication, chemical pollution and mechanical disturbance of the sea bottom are the major anthropogenic pressures determining changes in macrofauna abundance, distribution and species composition (McQuatters-Gollop et al., 2009; Van Hoey et al., 2010; Rice et al., 2012). Among those, eutrophication is often emphasized as a particularly large-scale driving force of ecosystem changes, having multiple indirect effects and therefore not being easily quantifiable by direct measurements (Van Hoey et al., 2010). Therefore, detection of eutrophication effects relies mostly on the sensitivity of selected indirect measurements and synoptic indicators (such as benthic indices).

Many studies have aimed to test and validate benthic indicators, applying different analytical frameworks and statistical approaches. For instance, the responsiveness of the BENTIX index (Simboura and Zenetos, 2002) to water quality parameters (dissolved oxygen, particulate and total organic carbon) was assessed using linear regression. Factorial analysis was used by Muxika et al. (2007) when validating benthic quality assessment performed with the AMBI Index (Borja et al., 2000). Diaz et al. (2004) assessed the functionality of 64 benthos-related indices applying qualitative comparison based on a comprehensive literature review.

Among different frameworks suggested for quality analysis of GES indicators, there is still little agreement on a uniform approach for a robust and standardized selection of appropriate metrics (Mazik et al., 2010; HELCOM, 2012). Here, we demonstrate the application of Signal Detection Theory (SDT) to identify and quantify the indicator response to a particular anthropogenic pressure. This method has been extensively used in medical studies, but has also been considered for ecological application (Murtaugh, 2006; Hale and Heltshe, 2008). In the current study, we assess the specificity and sensitivity of the Benthic Quality Index (BQI), its response to the eutrophication pressure, and its performance in relation to the soft-bottom habitats affected by estuarine water outflow.

2. Material and methods

2.1 Study area

The performance of the BQI was assessed in relation to the soft-bottom habitats in the Lithuanian coastal zone, south-eastern Baltic Sea (Fig. 1). Due to high wave exposure there is no oxygen deficiency in the near-bottom layer. Salinity in the study area varied from 6.3 to 7.4 ‰ outside the plume and decreased down to 3.3‰ in the areas exposed to a freshwater outflow from the Curonian Lagoon (the plume zone). Approximately 60 different benthic macrofauna species have been reported in this area (Olenin et al., 1996). Hard-bottom communities are dominated by the blue mussel *Mytilus edulis* and the barnacle *Amphibalanus improvisus*, whereas sandy bottoms are dominated by the spionid polychaetes *Pygospio elegans* and *Marenzelleria* spp. or the bivalve *Macoma baltica* (Bubinas and Vaitonis, 2003; Olenin and Daunys, 2004). Eutrophication is considered to be one of the main pressures affecting water quality in the study area (Olenin and Daunys, 2004).

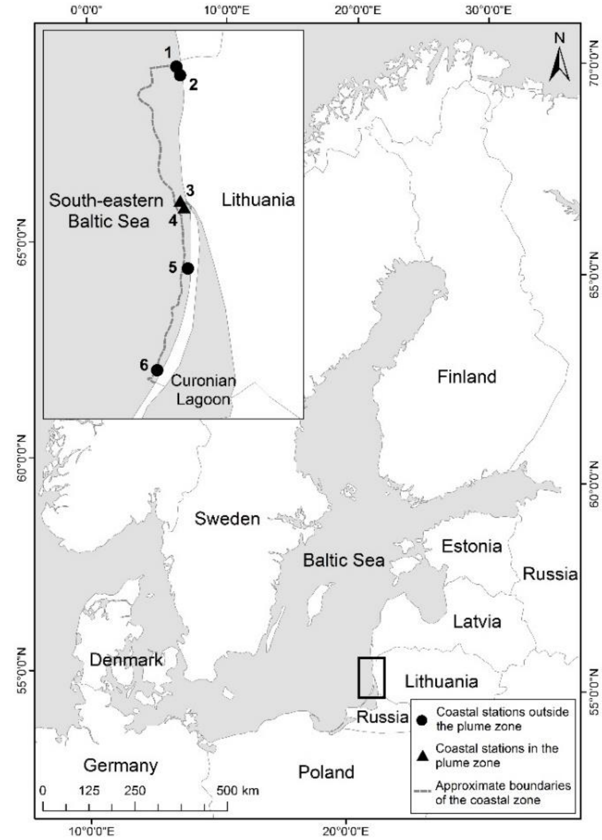


Fig. 1. Study area and sampling sites in the south-eastern part of the Baltic Sea. Black triangles denote sampling stations exposed to the reduced salinity conditions due to the freshwater outflow from the Curonian Lagoon (the plume zone); black dots – sampling stations outside the plume zone. Dashed lines indicate the approximate boundaries of the coastal zone and correspond to the 20 m isobaths.

2.2 Data collection

A long-term (May–September samplings between 1984 and 2012) benthic macrofauna data set covering six monitoring sites (Fig. 1) was used for assigning the species sensitivity values ($ES_{50\text{-}0.05}$), as described by Leonardsson et al. (2009). For the BQI calculation and responsiveness analysis, data (2005–2011) on macrofauna diversity and abundance (ind/m^2), and summer averages (June–August) of total phosphorus (TP mg/l), total nitrogen (TN mg/l) and chlorophyll-*a* (chl-*a* µg/l) concentrations were used. These parameters were chosen as "direct measures" of eutrophication, suggested among others within the MSFD (Ferreira et al., 2011).

Benthic samples were collected from the soft-bottom habitats at depths ranging from 13 to 20 m, sieved on-site through a 0.5 mm mesh and processed according to the standard HELCOM recommendations (COMBINE manual). Data on TP and TN were collected as part of the national monitoring programme (unpublished data, Environment Protection Agency), and chl-*a* data were retrieved from the MEdium Resolution Imaging Spectrometer (MERIS), the ENVISAT satellite of the European Space Agency.

The final data set used for the analysis consisted of 77 samples collected from six locations (Fig. 1) within the coastal zone.

2.3 BQI index calculations

When testing the responsiveness of the BQI to the eutrophication pressure (expressed by TP, TN and chl-*a* concentrations), a one-year lag was applied for the index values in respect of pelagic parameters. Instant effects (no lag) were less likely in our study due to the timing of pelagic and benthic samplings (June–August and May–September respectively). One-year lag was also supported by the best statistical response using multiple linear regression (r=0.30, p=0.08) of the BQI to environmental variables compared to no or two-year lag applications (r=0.06, p=0.80 and r=0.04, p=0.86 respectively).

Since the original version of the BQI (Rosenberg et al., 2004) is known to be sampling effort dependent (Fleischer et al., 2007), the adjusted calculation was applied (Fleischer and Zettler, 2009)

$$BQI = \left(\sum_{i=1}^{n}\left(\frac{A_i}{A_{tot}} \times ES50_{0.05i}\right)\right) \times \log\left(ES_{50}+1\right) \times \left(1-\frac{5}{5+A_{tot}}\right) \quad (1)$$

In the above equation, *n* denotes the observed species number. A_i stands for the abundance of the species *i* (ind m^{-2}) and A_{tot} is the sum of all individuals (ind m^{-2}). Finally, $ES50_{0.05i}$ is the sensitivity/tolerance value for the species *i* and ES_{50} denotes the estimated species number among 50 randomly picked individuals within a square metre (Hurlbert Index). The sensitivity value of a species was set to the 5^{th} percentile of the ES_{50} ($ES50_{0.05i}$) in the samples where the species was present.

2.4 Signal Detection Theory

According to SDT, the sensitivity and specificity of an indicator can be calculated according to four possible outcomes – hits (correct interpretation of a true response – true positives), misses (inability to detect a true response – false negatives), false alarms (false detection of a response – false positives) and correct rejections (correctly interpreted missing response – true negatives) – given that the target condition ("gold standard") is known. Receiver operating characteristic (ROC) curves provide a visual tool for assessing the accuracy of an indicator, by plotting the probability of the true positives (sensitivity) against the probability of the true negatives (specificity). The area under the ROC curve (AUC) can be used as a measure of the indicator response. A perfect indicator should have an AUC of 1, whereas 0.5 is a measure of a non-informative indicator (Murtaugh, 1996). In ecological studies, AUC values ≥0.8 are considered to indicate an excellent and ≥0.7 an acceptable response (Hale and Heltshe, 2008).

The predictive ability of the indicator is described by the positive predictive value (PPV: the probability of the true positives) and the negative predictive value (NPV: the probability of the true negatives) (Murtaugh, 1996). The PPV and NPV values vary, according to the prevalence of the target values in the analysed parameter (values at or above the good water quality threshold, as defined for this study). For example, at low prevalence of the target values a correct (true positive) response will only be attained with an accurate indicator, implying a low rate of false positives (Swets et al., 2000). Thus, by using PPV and NPV, the probability of getting a correct response can be evaluated against the risks of making wrong decisions. This approach can be used to set indicator thresholds for distinguishing impacted sites from undisturbed ones.

To test and verify the response of the BQI using ROC curves, the calculated values were related to gold standards based on TP, TN and chl-*a* concentrations. Since the quality class threshold between “good” and “moderate” status, *sensu* the Water Framework Directive (WFD; European Commission 2000/60/EC), is critical for distinguishing between substantial deviation and the natural range of the indicator values, it was applied for setting the gold standard (or target) values in the current SDT analysis (Table 1).

Table 1

Good/moderate status thresholds defined for the Lithuanian coastal area (WFD Lithuania Surface water bodies methodological material, 2009) and applied in the current STD analysis

Eutrophication parameter	**Outside the plume zone**	**Within the plume zone**
Chl-*a* concentration (μg/l)	≤4.8	≤25.7*
TP concentration (mg /l)	≤0.026	≤0.026**
TN concentration (mg g/l)	≤0.25	≤0.25**

* salinities <4‰

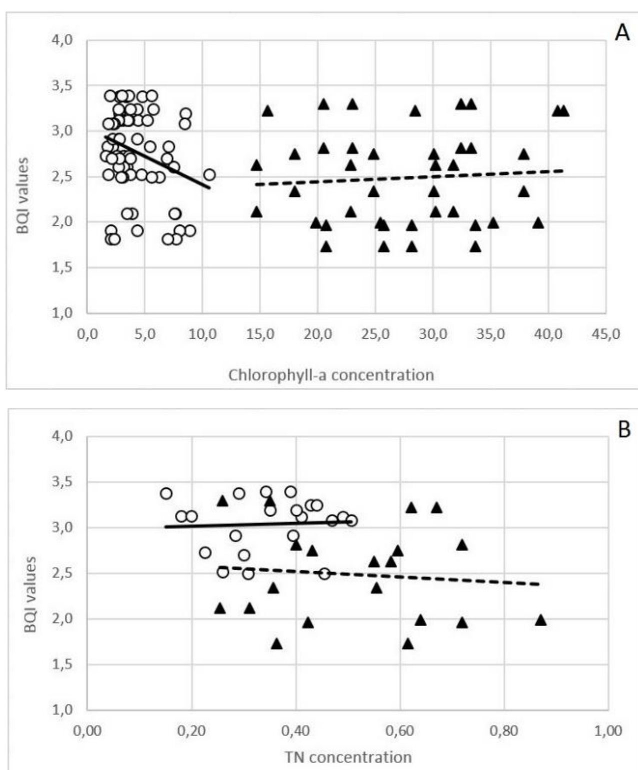
** salinities >4‰

2.5 Statistical analyses

Linear regression was applied to relate calculated BQI values to the environmental parameters (chl-a, TP, TN concentrations). The Bonferroni α-correction for α was applied for multiple pairwise tests. The Primer software package (Clarke and Warwick, 2001) was used for calculation of the Hurlbert Index (ES_{50}). The graphical visualizations (including ROC curves), threshold estimations and analyses were performed in the R v3 statistical computing environment (R-project 2014).

3. Results

The calculated BQI values ranged from 1.7 to 3.4 with no apparent difference between plume area and the rest of the coastal zone. The average chl-*a* and TN concentrations were significantly higher within the plume zone than outside the plume zone (chl-a: 27.5±1.1 µg/l and 4.1±0.3 µg/l respectively, $t = -25.018$, $p = 0.0001$; TN: 0.51±0.04 mg/l and 0.35±0.02 mg/l respectively, $t = -3.783$, $p = 0.0006$). This is supported by a significant negative relationship between salinity and TN concentrations in the plume ($r = -0.72$, $p < 0.001$, salinity range between 3.3 and 7.1 ‰), while outside the plume this relationship was negligible ($r = -0.02$, $p < 0.001$, salinity range between 6.3 and 7.4 ‰). TP concentrations were similar within and outside the plume with no significant differences between average values (0.038±0.002 mg/l and 0.034±0.003 mg/l respectively) and a very weak negative relationship with salinity.



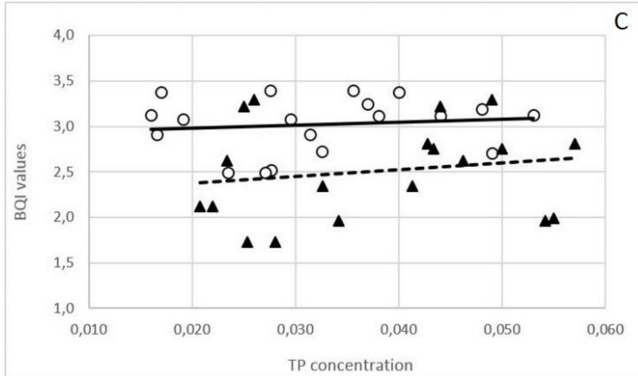


Fig. 2. BQI values *versus* chl-*a* concentrations (A), TN (B) and TP (C) with fitted linear model trend lines for the samples taken outside the plume (white dots, solid line) and within the plume zone (black triangles, dotted line).

For BQI validation, its relationships with chl-*a*, TN and TP summer concentrations were first analysed applying linear regression. When partitioning the plume effect (relating the BQI values to eutrophication parameters separately for sampling sites within and outside the plume area), a statistically significant negative correlation with chl-*a* concentrations ($r = -0.27$; $p = 0.02$) was revealed outside the plume zone only, while no significant relationships were detected for TN and TP either within or outside the plume zone. In general, the analysed eutrophication parameters had higher variability within the plume area (chl-*a* concentration range was between 14.7 and 41.3 µg/l, and TN and TP concentration ranges were 0.25–0.87 mg/l and 0.021–0.057 mg/l respectively) compared with their values outside the plume zone (chl-*a* concentration range was 1.7–10.5 µg/l, and TN and TP concentration ranges were 0.15–0.51 mg/l and 0.016–0.065 mg/l respectively) (Fig. 2).

The SDT analysis was performed separately for the studied coastal areas. In accordance with the AUC classification by Hale and Heltshe (2008), an acceptable BQI response (AUC = >0.70) was revealed to all analysed eutrophication parameters measured outside the plume zone. Within the plume zone, however, the index response to chl-*a* and TP concentrations was qualified as poor, but excellent for TN concentration (Fig. 3).

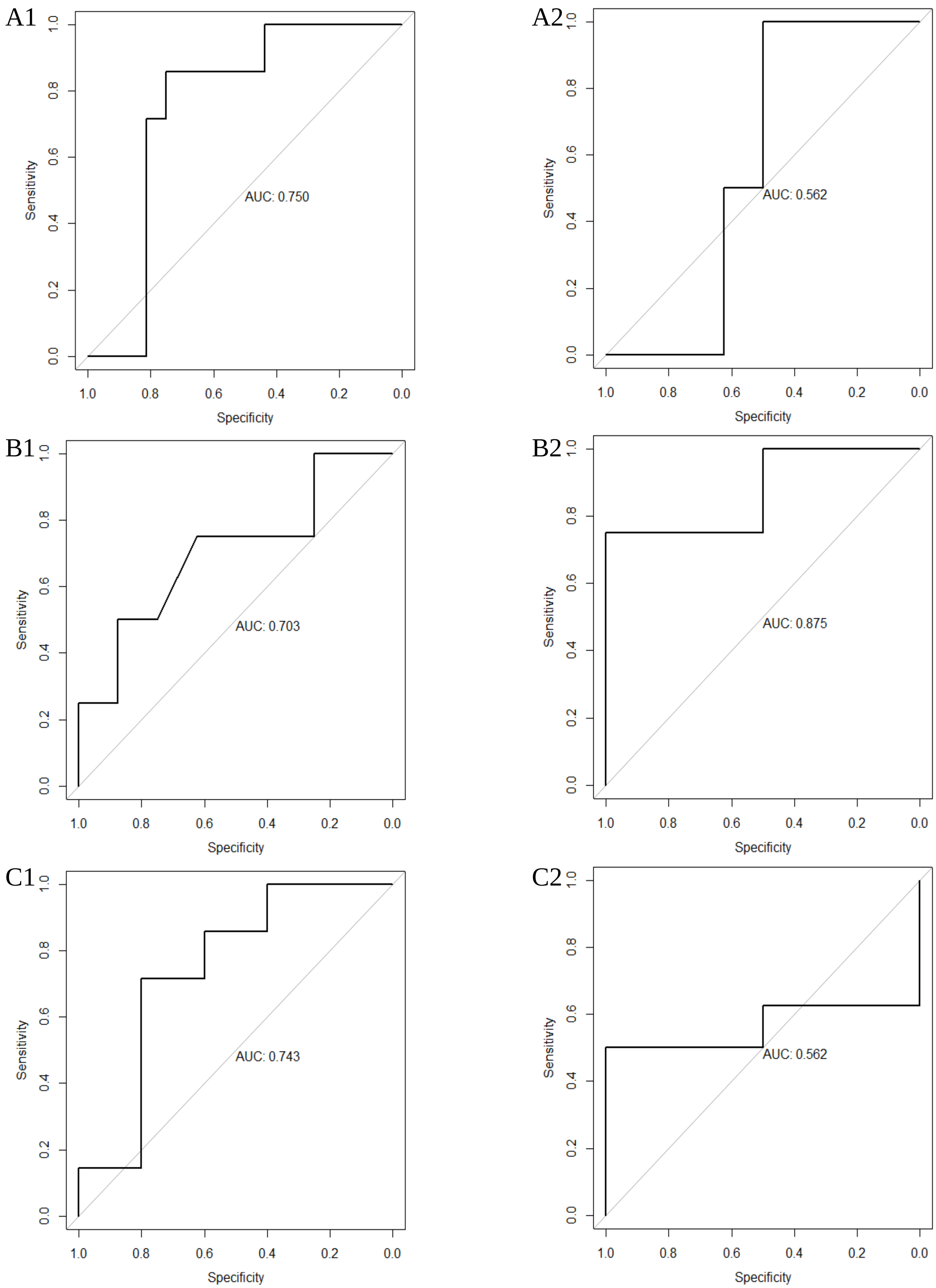


Fig. 3. ROC (receiver operating characteristic) curves for BQI response to chl-*a* (A), TN (B) and TP (C) concentrations in the study area outside the plume zone (left column) and within the plume zone (right column).

The relation between the BQI values and the chl-*a* concentrations outside the plume zone was used for setting the threshold between “acceptable” and “unacceptable” status of water quality (Fig. 4). BQI thresholds were assigned at different specificity and sensitivity levels.

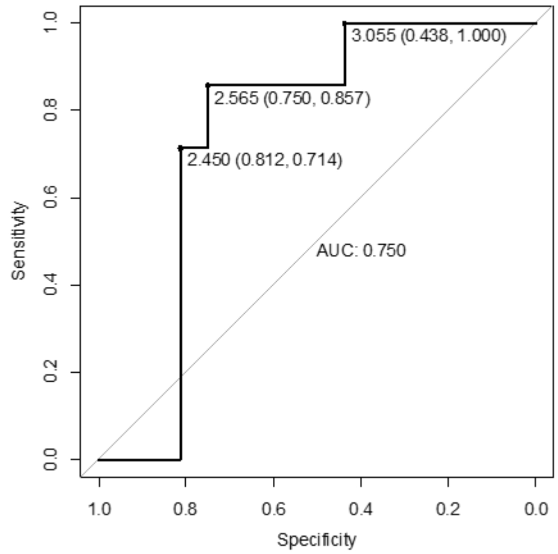


Fig. 4. ROC (receiver operating characteristic) curves for the BQI, as a response to the chl-*a* concentration. The steps denote proposed threshold values (strict – 2.45, the most accurate – 2.56 and lenient – 3.05). Numbers in brackets indicate specificity and sensitivity values respectively.

The most accurate BQI threshold, according to the sum of sensitivity and specificity values estimated from the index response to chl-*a* (i.e. ROC curves), was 2.56 (specificity and sensitivity 0.75 and 0.86 respectively; Fig. 4). In our data set, the prevalence of the chl-*a* values falling within the target range (between the good and moderate water quality classes) was 0.69 (16 out of 23 samples). At this prevalence, the most accurate BQI response showed an ability to correctly identify “acceptable” conditions in 89% of cases (positive predictive value, PPV) and “unacceptable” conditions in 68% of cases (negative predictive value, NPV) (Fig. 4, Table 2).

Table 2

Suggested BQI thresholds based on the response to chl-*a* concentrations outside the plume zone, with corresponding estimates of prevalence, specificity, sensitivity, PPV and NPV (based on SDT approach)

BQI thresholds for chl-*a* concentration values	Prevalence of the target chl-*a* values (between good and moderate conditions)	Specificity	Sensitivity	PPV (%)	NPV (%)
2.45 – strict	0.69	0.81	0.71	90	55
2.56 – the most accurate	0.69	0.75	0.86	89	68
3.05 – lenient	0.69	0.44	1.00	80	100

In a healthy environment, an assessment should rely more on NPV rather than on PPV and vice versa. For instance, when applying a strict threshold at 2.45 (BQI boundary between the good and moderate environmental status classes), the PPV is higher (90%) and NPV is lower (55%) compared to a threshold set at the most accurate BQI response (2.56). A lenient threshold at 3.05 results in a comparatively low PPV and high NPV (80% and 100% respectively) (Table 2).

4. Discussion

The BQI is one of the most widely used multimetric indices for macrofauna status assessment (Rosenberg et al., 2004; Fleischer and Zettler, 2009; Leonardsson et al., 2009). Although designed for application in marine areas, it is also considered to be suitable for different environments provided that the assigned species' tolerance/sensitivity values are based on individual data sets and are site-specific (Zettler et al., 2007). The index is assumed to be ecosystem relevant and reproducible since it has been tested and validated in different marine ecosystems with varying environmental conditions, however its performance can be affected by the salinity gradient and the presence of invasive species (Labrune et al., 2006; Zettler et al., 2007; Zaiko and Daunys, 2015).

Our results revealed an acceptable BQI response to the analysed eutrophication parameters for coastal waters. These results support the applicability of the BQI for benthic quality assessment in relation to nutrient/organic pollution (eutrophication pressure) in the exposed coastal areas of the brackish Baltic Sea. However, the response was not detected with the traditional statistical approach (linear regression). The effect of organic pollution on benthic communities is unlikely to be straightforward and therefore difficult to measure. When testing an indicator's responsiveness, ideally the assessment should be performed along the gradient of the selected pressure (i.e.

eutrophication in our case), excluding any untargeted disturbances (noise effects). This is, however, an unlikely case when working with typical field data and particularly with those from coastal areas, where multiple natural and anthropogenic factors are often simultaneously present.

In the Baltic Sea, eutrophication constitutes one of the most important pressures affecting different ecosystem components – from phytoplankton to the benthic communities (HELCOM, 2009). Many parameters have been proposed for measuring the eutrophication effects, e.g. optical water column properties, oxygen concentration, frequency of algae blooms, chlorophyll-*a* and nutrient concentrations. Only a few of them could be used for the pressure-response analysis due to the lack of consistent long-term observations. An increase in nutrient concentrations directly affects phytoplankton development and chl-*a* concentration. During succession, the phytoplankton biomass turns to organic material and becomes a food supply for the benthic communities in the case of transfer of material to the near-bottom layer or sediment as supported by vertical flux. Previous studies demonstrate that relationships between macrozoobenthos biomass and nutrients (TN and TP concentrations) were little affected by coastal exposure, and benthic invertebrates were more sensitive to changing TN concentrations in the shallower areas than in the deeper ones (Kotta et al., 2007). The indirect eutrophication effects such as altered species diversity or the proportion of tolerant and sensitive species do not assert instantly and may accumulate over time. They may also emerge later depending on the intensity of the impact and involved mechanisms (changed reproduction rate, modified feeding activity, increased physiological stress etc.) (Kotta et al., 2007; Grall and Chauvaud, 2002; Heip, 1995). As a result, a certain lag period is typically involved in relationships between benthic and pelagic parameters (e.g. Snickars et al., 2014).

Signal detection theory provides an appropriate approach for determining the underlying response. As a non-parametric method it is insensitive to general statistical assumptions, but nonetheless is able to provide estimates of indicator sensitivity and specificity as well as its predictive value. This method proved to be effective in uncovering the BQI response to eutrophication parameters in our case study. However, we found some inconsistencies in the BQI response to eutrophication parameters assessed by SDT in two cases (i.e. poor response to chl-*a* and TP concentrations within the plume zone). The plume zone is characterized by a strong spatial and temporal salinity gradient and organic enrichment; however, their effects on the macrobenthic community structure may not necessarily coincide. In most cases, low salinity is the driving force of the macrofauna distribution pattern and community composition within the plume zones (e.g. Boesch, 1977; Ysebaert et al., 1993; Bonsdorff, 2006).

Therefore, the response of benthic organisms to the indirect eutrophication parameters such as chl-a, TN or TP concentrations might be partly masked. Also, highly eutrophied areas with a low-salinity regime are known to be predominantly N-limited (Tamminen et al., 2007), hence a stronger BQI response to TN concentrations is more likely in the plumes.

The overarching purpose of any environmental indicator is to distinguish between healthy and degraded environments and provide a scientifically based reasoning for undertaking appropriate measures to improve the ecological status. The application of the SDT approach can assist in assessing the performance of candidate environmental metrics under particular conditions, setting the threshold values and evaluating water quality status in a robust and scientifically sound way. The most accurate index threshold suggested by SDT might not always be the preferred choice, as management effort may be advisable in some cases, when degradation is less pronounced and natural recovery is still feasible. On the other hand, an environmental manager assessing the status of particularly valuable or protected areas might prefer the lower risk of overlooking deterioration and therefore will need to maximize NPV values and set a lenient threshold for the index. If the BQI is assessed in a largely affected area, the positive predictions will be more accurate than the negative ones, hence maximized PPV values and a stricter threshold for the index are advisable. Considering these aspects would help to support the adequate management effort and appropriate remediation measures on site (Hale and Heltshe, 2008).

SDT provides a practical tool to validate indicator thresholds and select good environment status (GES) boundaries for a particular area. Based on the SDT analysis results, one could decide whether an indicator is representative enough for detecting the particular pressure. Depending on the targets set, information retrieved from the SDT analysis can be used for designing the monitoring programme and answering practical ecological and management questions, e.g. how dense the sampling network should be to detect the pressure and assess the environmental status in light of the specific conditions, potential noise factors and uncertainties involved.

5. Conclusions

Although the traditional data exploration methods showed a weak or no relation between the BQI and the selected eutrophication parameters, SDT indicated a clear BQI response to the eutrophication pressure in the studied area. The response was affected by the freshwater outflow from the Curonian Lagoon, though. Signal detection theory (ROC curves, PPV and NPV approach) can be proposed as a standardized method to assess the responsiveness of an indicator to a particular pressure and set appropriate threshold values for the environmental status assessment. The thresholds, however, should be adjusted for a particular area or ecosystem to fit the environmental and management context.

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References

1. Bubinas A., Vaitonis G., 2003. The analysis of the structure, productivity, and distributions of zoobenthocenoses in the Lithuanians economic zone of the Baltic sea and the importance of some benthos species to fish diet. Acta Zoologica Lituanica, Volumen 13, Numerus 2, ISSN 1648-6919.
2. Boesch, D. E., 1977. A new look at the zonation of benthos along the estuarine gradient. In Ecology of marine benthos, (B. C. Coull, ed.) pp. 245-266, Univ. of South Carolina Press, Columbia.
3. Bonsdorff E., 2006. Zoobenthic diversity-gradients in the Baltic Sea: Continuous post-glacial succession in a stressed ecosystem. Journal of Experimental Marine Biology and Ecology 330, 383 – 391.
4. Borja, A., Dauer, D.M., 2008. Assessing the environmental quality status in estuarine and coastal systems: comparing methodologies and indices. Ecological Indicators 8, 331-337.

5. Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine Pollution Bulletin 40, 1100–1114.
6. Buhl-Mortensen, L., Aure, J., Oug, O., 2009. The Response of Hyperbenthos and Infauna to Hypoxia in Fjords Along The Skagerrak: Estimating Loss of Biodiversity Due to Eutrophication. In: Moksness., E., Stotterup, E., Dahl, J. (Eds.), Integrated Coastal Zone Management. Wiley-Blackwell Publ, UK, pp. 79–96.
7. Clarke, K. R., Warwick R. M., 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E, Plymouth, UK.
8. Dauvin, J.C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. Marine Pollution Bulletin 55, 215–224.
9. Dauvin J.C, Ruellet, T., 2009. The estuarine quality paradox: Is it possible to define an ecological quality status for specific modified and naturally stressed estuarine ecosystems? Marine Pollution Bulletin 59, 38-47.
10. Diaz, R. J., Solan, M., Valente, R. M., 2004. A review of approaches for classifying benthic habitats and evaluating habitat quality. Journal of Environmental Management 73, 165-181.
11. Elliott, M., 2011. Marine science and management means tackling exogenic unmanaged pressures and endogenic managed pressures – a numbered guide. Marine Pollution Bulletin 62, 651-655.
12. European Commision (2000). Water Framework Directive; WFD; 2000/60/EC (2000).
13. European Commision (2008). Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for Community actions in the field of marine environmental policy (Marine Strategy Framework Directive).
14. Ferreira, J. G., Andersen, J. H., Borja, A., Bricker, S. B., Camp, J., Cardoso da Silva, M., Garces, E., Heiskanen, A.-S., Humborg, C., Ignatiades, L., Lancelot, C., Menesguen, A., Tett, P., Hoepffner, N., Claussen, U., 2011. Overview of eutrophication indicators to assess environmental status within the European Marine Strategy Framework Directive. Estuarine, coastal and shelf science 93, 117-131.
15. Fleischer, D., Zettler M. L., 2009. An adjustment of benthic ecological quality assessment to effects of salinity. Marine Pollution Bulletin 58, 351–357.

16. Fritzsche, D., Oertzen von J. A., 1995. Metabolic responses to changing environmental conditions in the brackish water polychaetes Marenzelleria viridis and Hediste diversicolor. Marine Biology 121:693-699.
17. Hale, S.S., Heltshe, J. F., 2008. Signals from the benthos: Development and evaluation of a benthic index for the nearshore Gulf of Maine. Ecological indicators 8, 338-350.
18. HELCOM., 2009. Eutrophication in the Baltic Sea – An integrated thematic assessment of the effects of nutrient enrichment and eutrophication in the Baltic Sea region. Baltic Sea Environment Proceedings No. 115B.
19. HELCOM., 2012. Development of a set of core indicators: Interim report of the HELCOM CORESET project. PART A. Description of the selection process. Baltic Sea Environment Proceedings No. 129A.
20. Heip C., 1995. Eutrophication and zeoobenthos dynamics. Ophelia, 41: 113-136.
21. ICES., 2013. Report of the working group on the ecosystem effects of fishing activities (WGECO). ICES, Copenhagen, Denmark.
22. Ysebaert T., Meire P., Buijs J., 1993. The benthic macrofauna along the esturarine gradiens of the schelde estuary. Nehterlands Journal of Aquatic ecology, 27 (2-4), 327-341.
23. Ysebaert, T., Herman, P. M. J., 2002. Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. Marirne Ecology Progress Series, Vol. 244: 105-124.
24. Kershner, J., Samhouri, J. F., James, C. A., Levin, P. S., 2011. Selecting indicator portfolios for marine species and food webs: a puget sound case study. PLoS One 6, e25248.
25. Labrune, C., Amouroux, J.M., Sarda, R., Dutrieux, E., Thorin, S., Rosenberg, R., Gremare, A., 2006. Characterization of the ecological quality of the coastal Gulf of Lions (NW Mediterranean). A comparative approach based on three biotic indices. Marine Pollution Bulletin 52, 34-47.
26. Leonardsson, K., Blomqvist, M., Rosenberg, R., 2009. Theoretical and practical aspects on benthic quality assessment according to the EU-Water Framework Directive – examples from Swedish waters. Marine Pollution Bulletin 58, 1286–1296.
27. Programme of HELCOM, Part C. Manual for Marine Monitoring in the COMBINE. Programme for monitoring of eutrophication and its effects. Annex C-8.

28. Mazik, K., Boyes, S., McManus, E., Ducrotoy, J.-P., Rogers, S., Elliott, M., 2010. Healthy & biologically diverse seas evidence group technical report series: Evaluation and gap analysis of current and potential indicators for sediment habitats.
29. McQuatters-Gollop, A., Gilbert, A. J., Mee, L. D., Vermaat, J. E., Artioli, Y., Humborg, C., Wulff, F., 2009. How well do ecosystem indicators communicate the effects of anthropogenic eutrophication? Estuarine, coastal and shelf science 82, 583-596.
30. Muxika, I., Borja A., Bald J., 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Marine Pollution Bulletin 55, 16–29.
31. Murtaugh, P. A., 1996. The statistical evaluation of ecological indicators. Ecological Applications 6, 132–139.
32. Niemeijer, D., de Groot, R.S., 2008. A conceptual framework for selecting environmental indicator sets. Ecological Indicators 8, 14-25.
33. Olenin, S., Daunys D., Labanauskas V., 1996. Classification principles of the Lithuanian coastal biotopes. Annals of Geography, Vilnius 29, 218-231.
34. Olenin, S., Daunys D., 2004. Coastal typology based on benthic biotope and community data: The Lithuanian case study Coastline Reports 4, ISSN 0928-2734:65 – 83.
35. Olenina, I., 1997. Phytoplankton and its development in the South-Eastern coastal Baltic and the Curonian lagoon, Doctoral dissertation thesis (in Russian). Vilnius, 161 pp.
36. R-project. 2014. R: A language and environment for statistical computing. *in*. R Foundation for Statistical Computing, Vienna, Austria, [ISBN 3-900051-07-0], http://www.R-project.org.
37. Rice, J., Arvanitidis, C., Borja, A., Frid, C., Hiddink, J. G., Krause, J., Lorance, P., Ragnarsson, S. A., Skold, M., Trabucco, B., Enserink, L., Norkko, A., 2012. Indicators for sea-floor integrity under the European Marine Strategy Framework Directive. Ecological Indicators 12, 174-184.
38. Rice, J. C., Rochet, M. J., 2005. A framework for selecting a suite of indicators for fisheries management. ICES Journal of Marine Science 62, 516-527.
39. Rombouts, I., Beaugrand, G., Fizzala, X., Gaill, F., Greenstreet, S.P.R., Lamare, S., Le Loc'h, F., McQuatters-Gollop, A., Mialet, B., Niquil, N., Percelay, J., Renaud, F., Rossberg, A.G., Feral, J.P., 2013. Food web indicators under the Marine Strategy Framework Directive: from complexity to simplicity? Ecological Indicators 29, 246-254.
40. Rosenberg, R., Blomqvist, M., Nilsson, H. C., Cederwall, H., Dimming, A., 2004. Marine quality assessment by use of benthic species – abundance distributions: a proposed new protocol

within the European Union Water Framework Directive. Marine Pollution Bulletin 49, 728 – 739.

41. Simboura, N., Zenetos, A., 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems including a new biotic index. Mediterranean Marine Science, 3 (2), 77–111.
42. Snickars, M., Weigel B., Bonsdorff E., 2014. Impact of eutrophication and climate change on fish and zoobenthos in coastal waters of the Baltic Sea. Marine Biology, 162:141–151.
43. Swets, J. A., Dawes, R. M., Monahan, J., 2000. Better decisions through science. Scientific American, 283, 82-87.
44. Tagliapietra, D., Sigovini, M., Ghirardini, A.V., 2009. A review of terms and definitions to categorise estuaries, lagoons and associated environments. Marine and Freshwater Research 60, 497–509.
45. Tamminen, T., Andersen T., 2007. Seasonal phytoplankton nutrient limitation patterns as revealed by bioassays over Baltic Sea gradients of salinity and eutrophication. Marine Ecology Progress Series, Vol. 340: 121–138.
46. Van Hoey, G., Borja, A., Birchenough, S., Buhl-Mortensen, L., Degraer, S., Fleischer, D., Kerckhof, F., Magni, P., Muxika, I., Reiss, H., Schroder, A., Zettler, M. L., 2010. The use of benthic indicators in Europe: from the Water Framework Directive to the Marine Strategy Framework Directive. Marine Pollution Bulletin 60, 2187-2196.
47. Wasmund, N., Andrushaitis, A., Lsiak-Pastuszak, E., Müller-Karulis, B., Nausch, G., Neumann, T., Ojaveer, H., Olenina, I., Postel, L., Witek, Z., 2001. Trophic status of the South-Eastern Baltic Sea: a comparison of coastal and open areas. Estuarine, Coastal and Shelf Science 56, 849-864.
48. WFD report, Lithuania transitional and coastal waters status, an affecting factors and water condition improvement measures, Surface water bodies methodological material, 2009.
49. Zaiko, A., Daunys, D., 2015. Invasive ecosystem engineers and biotic indices: giving a wrong impression of water quality improvement? Ecological Indicators 52, 292–299.
50. Zettler, M.L., Schiedek, D., Bobertz, B., 2007. Benthic biodiversity indices versus salinity gradientin the southern Baltic Sea. Marine Pollution Bulletin, 55:258–270.