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Benthic biodiversity indices versus salinity gradient in the southern Baltic Sea

Michael L. Zettler *, Doris Schiedek, Bernd Bobertz

Baltic Sea Research Institute, Seestr. 15, D-18119 Rostock, Germany

Abstract

The need to assess the environmental status of marine and coastal waters according to the EU Water Framework Directive (WFD) encouraged the design of specific biotic indices to evaluate the response of benthic communities to human-induced changes in water quality. In the present study three of these indices, the traditional Shannon Wiener Index (H′) and the more recently published AMBI (AZTI′ Marine Biotic Index) and BQI (Benthic Quality Index), were tested along a salinity gradient in the southern Baltic Sea. The comparison of the three indices demonstrates that in the southern Baltic Sea the ecological quality (EcoQ) classification based on macrozoobenthic communities as indicator greatly depends on the biotic index chosen. We found a significant positive relation between species number, H′, BQI and salinity resulting in EcoQ status of "Bad", "Poor" or "Moderate" in areas with a salinity value below 10 psu. The AMBI was less dependent on salinity but appear to partly overestimate the EcoQ status. Presently none of these biotic indices appear to be adjusted for application in a gradient system as given in the southern Baltic Sea. A potential approach describing how to overcome this limitation is discussed.

Keywords: Southern Baltic Sea; Macrozoobenthos; Biotic indices; Ecological quality; Salinity gradient; Water Framework Directive; Oxygen depletion

1. Introduction

The development of biological indicators as a tool for the assessment and hence protection of biological diversity in European coastal and marine ecosystems has been advanced due to the implementation of the Habitats Directive and the Water Framework Directive (WFD). Benthic invertebrates are often used as bioindicators to detect and monitor environmental changes, because of their rapid responses to natural and/or anthropogenic caused stress (e.g., Pearson and Rosenberg, 1978; Grall and Glémarec, 1997; Simboura and Zenetos, 2002; Perus et al., 2004). Benthic species are relatively long-living sessile organisms unable to avoid unfavourable conditions. In this way, they integrate water and sediment quality conditions over time and their presence/absence indicates temporal as well as spatial disturbances (Reiss and Kröncke, 2005). In the past years different biotic indices have been designed to assess the ecological quality of European coasts. In this respect, the Shannon–Wiener index H′ (Pielou, 1975), the BQI (Benthic Quality Index, Rosenberg et al., 2004) and the AMBI (Azti Marine Biotic Index, Borja et al., 2000) are among those indices generally used. The AMBI has been proposed for the assessment of the ecological status of estuarine and coastal waters, whereas the BQI were mainly designed for application in marine areas. The main purpose of all of them is to separate impacted sites from undisturbed (reference) sites (e.g., Borja et al., 2003; Muxika et al., 2005; Labrune et al., 2006). Their application, however, does not necessarily allow distinguishing between natural or man-induced disturbances and their natural variability both on temporal and spatial scales has to be assessed (Vincent et al., 2002).

In brackish water system such as the Baltic Sea two main environmental variables (salinity and oxygen supply) affect the composition of the benthic community and

* Corresponding author. Fax: +49 381 5197440.
E-mail address: michael.zettler@io-warnemuende.de (M.L. Zettler).

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species' abundance (e.g., Rönnberg and Bonsdorff, 2004). Benthic diversity differs from other coastal systems (e.g., North Sea) and the applicability of biodiversity indices has to be evaluated (Rosenberg et al., 2004).

The Baltic Sea, formed after the latest glaciation, is a young ecosystem continuously undergoing post-glacial successional changes (Jansson and Jansson, 2002). It is an enclosed, non-tidal ecosystem and has steep latitudal and vertical salinity gradients. The southern parts including the Belt Sea are closely connected to the Kattegat and Skagerak and show salinities between 25 and 30 psu. Within few 100 km east- or northwards the values drop down to 5 psu and, finally, in the northern part to more or less freshwater conditions. As a consequence, the number of marine species is significantly decreased or has been displaced by limnic species in the North and inner coastal waters (Bonsdorff, 2006). Even though the Baltic is a young ecosystem, species-poor and vulnerable to the threat of invasive marine and exotic species, both the strong gradient and the rapid change of salinity conditions especially in the southern Baltic inhibit an unhindered colonisation. As a result, the Baltic benthic fauna is still largely characterised by species with obviously opportunistic life history traits (Rumohr et al., 1996).

The salinity gradient is particularly pronounced in the transition zone ranging from the euhaline Skagerrak and Kattegat to the brackish Baltic Proper (down to 5 psu). Owing to the strong salinity reduction from West to East macrobenthic biodiversity decreases rapidly in the southern Baltic (Zettler and Röhner, 2004). Whereas in the Kiel Bight about 700 species occur, only ≈100 are present in the Pomeranian Bay. The rapid decline in the overall number of species along the Baltic Sea salinity gradient is illustrated by Bonsdorff (2006).

The objectives of the present study were to (i) use different biotic indices to assess macrozoobenthic diversity along a strong salinity gradient, (ii) compare different indices and their correlation to salinity and (iii) assess their sensitivity to severe impacts (e.g., temporal oxygen depletion). We compared the H′, BQI and AMBI at 625 stations located in the southern Baltic Sea, sampled during the last 10 years. The salinity range in the investigation area was 1.5–27.8 psu. Our work represents the first comparison of these biotic indices for the southern Baltic Sea and German coastal waters in particular and their applicability for the Water Framework Directive along this strong salinity gradient.

2. Material and methods

2.1. Study area

The investigation area has an expansion of 300 km in longitude and 150 km in latitude and is composed of different water bodies (Fig. 1). The Belt Sea extends from the Kiel Bight via Mecklenburg Bight to the Darss Sill and is regarded as a part of the transition zone between the Kattegat and the deep basins of the Baltic Proper. The first of these basins with water depths up to 50 m (Arkona Basin) borders to the Bornholm area in the East and to the

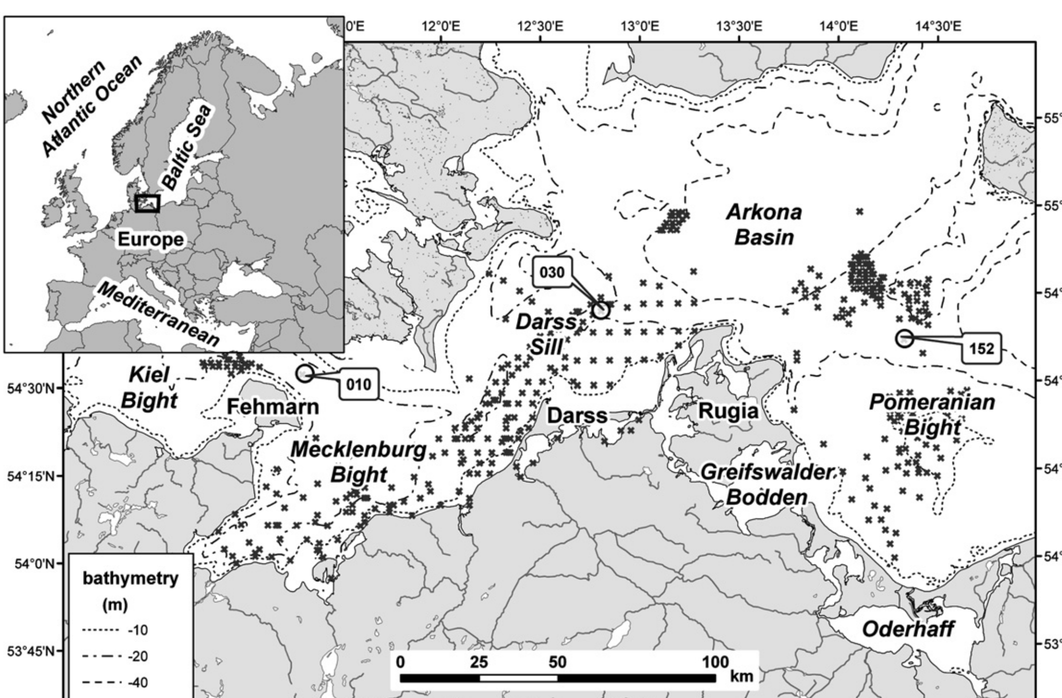

Fig. 1. Investigation area in the southern Baltic Sea. In total 625 stations (crosses) were sampled between 1995 and 2005, including three long term monitoring stations (010, 030, 152).

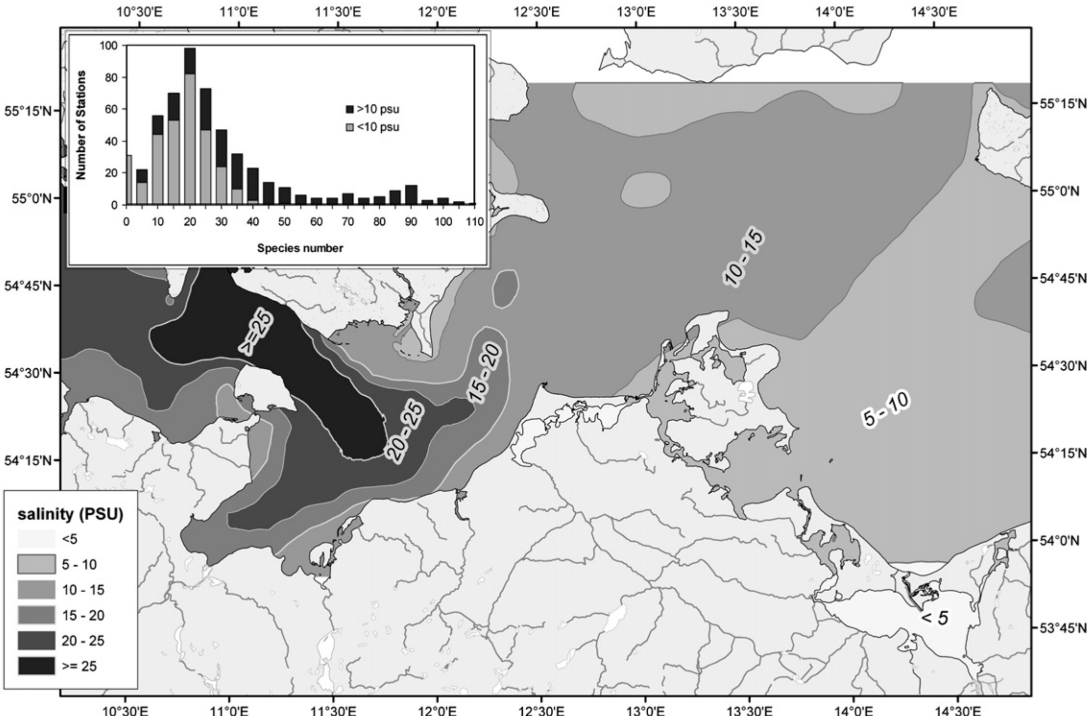

Fig. 2. Distribution map of the modeled mean bottom water salinity (period 1993–2003) in the southern Baltic Sea. The difference between highest and lowest measured values was 26.3 psu. The histogram indicates the occurrence of species at two different salinity ranges (< and >10 psu).

Pomeranian Bight in the South. The latter is a shallow water body with depths of 13 m in average and politically divided by the German–Polish border in the East. The whole investigation area covers an area of about 35.000 km^2.

In water depths <20 m the main sediment type consists of sand. The deeper areas, e.g., depths >20 in the Mecklenburg Bight or >35 m in the Arkona Basin, are characterised by muddy substrates. Gravel, stones and boulders are typical for underwater glacial banks or exposed shore lines. Whereas the narrow Little and Great Belt connect the Kattegat to the southern Belt Sea, the narrow Sound connects the Kattegat and the Arkona Basin directly. Due to these narrow connections, each slices through sills, the events of inflowing saline waters into the Baltic Sea are limited and occasionally with stagnation periods over weeks up to months. Along the German Baltic coast the salinity of the bottom waters ranges between about 25 psu in the West and 7 psu in the East (Fig. 2). A second strong gradient occurs from the offshore waters to the inner coastal waters and estuaries. Especially stations within the boddens south of Darss (with salinities between 1 and 8 psu) and the Greifswalder Bodden (3–7 psu) were taken into account.

2.2. Macrofauna data sets

Macrozoobenthic data compiled for this study comprise the southern Baltic Sea and in particular the German Baltic waters. The data sets analysed are based on 625 stations sampled by the Baltic Sea Research Institute during the past 10 years. Benthic samples were taken with a 0.1 m^2 van Veen grab. Depending on sediment composition, grabs of different weights were used. Three (or two) replicates of grab samples were taken at each station. Additionally a dredge haul (net mesh size 5 mm) was taken in order to obtain mobile or rare species. Exceptionally, in the shallow inner coastal waters a hand corer with an area of 78.5 cm^2 was applied. All samples were sieved through a 1-mm screen and animals were preserved in the field with 4% formaldehyde. For sorting in the laboratory, a stereo-microscope with 10–40× magnification was used. All macrofauna samples were identified to the lowest possible taxonomic level. The nomenclature was checked following the European Register of Marine Species (Costello et al., 2001). For the characterisation of the habitat (i.e., assessment of sediment structure or species on the sediment surface), an underwater video-system mounted on a sledge was used. The salinity of near bottom waters was measured by hand-held equipment in coastal waters or by means of a ship-based CTD-sensor in offshore waters.

2.3. Data analysis

A variety of diversity indices has been used in benthic ecology to assess the environmental quality and the effects of disturbances on benthic communities. In the present study, macrobenthic data were used for the computation of the Shannon–Wiener Index (H′) (Pielou, 1975), Azti Marine Biotic Index (AMBI) (Borja et al., 2000; Borja et al., 2003; Muxika et al., 2005) and the Benthic Quality

Index (BQI) (Rosenberg et al., 2004). The procedure how to calculate the indices are well described in these papers listed above and thus the formulas are not documented here.

The Primer package (Clarke and Warwick, 1994) was used for the analysis of the classical Shannon–Wiener Index and the Hurlbert Index (ES50). The latter is included in the first term of the BQI and reflects the number of species expected from a sub-sample of 50 individuals taken from the population of all the individuals present at a given station. Rosenberg et al. (2004) proposed to characterise the tolerance/sensitivity of a given species based on its $ES50_{0.05}$. This term is defined as the ES50 corresponding to 5% of the total abundance of this species within the studied area. For each species present in the investigation area the $ES50_{0.05}$ value was calculated and used for the computation of the BQI. The AMBI was calculated using the AMBI 3.0 program available on the web page http://www.azti.es according to the guidelines from the authors (Borja and Muxika, 2005).

The AMBI is based on the proportion of five ecological groups (EG) to which the benthic species are allocated (Borja et al., 2000). A list including >3400 benthic taxa and their assignment to the five ecological groups could be downloaded. Only few species present in our investigation area were not covered by this list. In these cases we used the following procedures. We either tried to reduce the taxonomic level to genus or family level, if the higher levels are assigned in the list (e.g., *Caprella septentrionalis* to *Caprella* sp. or *Cadlina laevis* to Chromodoridae) or we assigned "our species" to closely related species (e.g., *Balanus* sp. to *Semibalanus* sp.). If neither of both was possible, the species were ignored. Most of these changes did not have an influence on the calculation of the AMBI since their number was well below 20% and most of them did not belong to one of the five ecological groups in the AMBI library.

For all parameters tested (salinity, Shannon, BQI, AMBI), the Komolgorov–Smirnov fitting test rejected the normal distribution hypothesis with a significance level less than $p < 0.01$. The magnitude and direction of the association between the variables were tested by a Spearman Rho correlation.

2.4. EcoQ assessment

For H′ and AMBI, an absolute scale composed of five classes of EcoQ has been proposed (Vincent et al., 2002; Borja et al., 2004a,b). The EcoQ assessed with BQI is determined by taking the highest BQI value as a reference value and by defining five classes of equal size between 0 and this reference value (Rosenberg et al., 2004). In our investigation area, the BQI varied between 0.67 (bad ecological quality) and 24.49 (high ecological quality), therefore, we divided the stations into 5 groups between 0 and 25. All EcoQ status are summarised in Table 1.

Table 1
H′, AMBI and BQI classes associated with the different EcoQ status proposed for the Water Framework Directive

EcoQ	H′	AMBI	BQI[a]
High	$H' > 4$	$AMBI \leqslant 1.2$	$BQI \geqslant 20$
Good	$3 < H' \leqslant 4$	$1.2 < AMBI \leqslant 3.3$	$15 \leqslant BQI < 20$
Moderate	$2 < H' \leqslant 3$	$3.3 < AMBI \leqslant 4.3$	$10 \leqslant BQI < 15$
Poor	$1 < H' \leqslant 2$	$4.3 < AMBI \leqslant 5.5$	$5 \leqslant BQI < 10$
Bad	$H' \leqslant 1$	$AMBI > 5.5$	$BQI < 5$

[a] In this study the BQI varied between 0 and 25.

3. Results

After the analysis of 625 sampled stations, in total 389 taxa were observed in the southern Baltic Sea (Fig. 3). Polychaetes showed the highest biodiversity (102 species), followed by molluscs (93 species) and crustaceans (85 species). Diversity of other groups such as cnidarians, clitellates and bryozoans was clearly lower (19–37 species). Nemerteans , poriferans, ascideans and echinoderms were only found in species numbers <10 (Fig. 3). The miscellaneous group consists of single taxa of several other taxonomical groups (priapulids, kamptozoans, turbellarians, phoronids, insects, arachnids, pycnogonids). Uncountable species (like bryozoans and hydrozoans) were excluded from the computation of the indices. In order to exclude species occurring in a few samples only, the number of sample occasions a species must have been recorded was limited to $\geqslant 3$. The analysis of univariate and multimetric indices were conducted with 211 taxa.

The 30 most common species including lowest and highest $ES50_{0.05}$, respectively, are listed in Table 2. The neozoan polychaete *Marenzelleria neglecta* featured a value of 1.4, the lowest value found in the present study. With up to 60.000 ind. m^2 at some stations this species showed the highest abundance compared to all other species. *M. neglecta* is characteristic for species poor areas of inner coastal waters in the Baltic Sea (Zettler et al., 2002). The abundance of the ascidia *Ciona intestinalis*, an epibenthic species of hard bottoms, gravel or macrophytes in more saline areas, features an $ES50_{0.05}$ of 17.8, the highest value recorded in the present study. In more ubiquitous species (e.g., *Pygospio elegans*, *Macoma balthica*, *Mya arenaria*) the values were similar to those from the Kattegat/Skagerrak area whereas the more marine species (e.g., *Buccinum undatum*, *Corbula gibba*, *Astarte montagui*, *Laonome kroeyeri*) partly show very different values (Table 2). This emphasises the importance of area specific reference values when calculating the ES50.

3.1. Shannon–Wiener index (H′)

In the southern Baltic Sea the Shannon–Wiener index ranged from 0.06 to 4.81 (Fig. 4). In terms of EcoQ the whole range from "Bad" to "High" was covered. Most

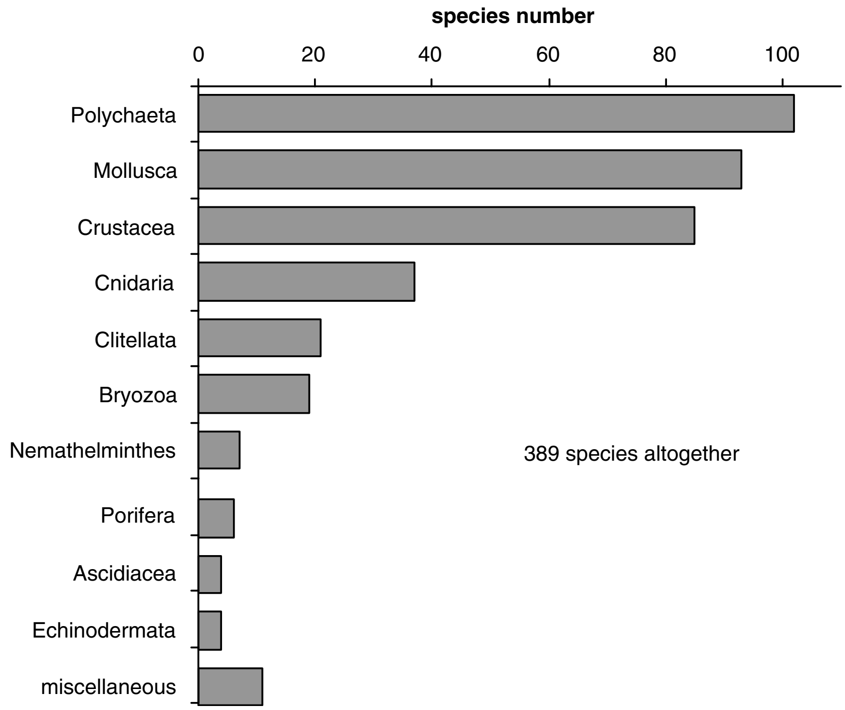

Fig. 3. Macrozoobenthos composition in the southern Baltic Sea based on 625 stations sampled between 1995 and 2005.

Table 2
$ES50_{0.05}$ including lowest and highest values of 30 common species in the southern Baltic Sea and the Kattegat/Skagerrak area

Species	Present study	Kattegat/Skagerrak[a]
Marenzelleria neglecta	1.4	
Gammarus tigrinus	2.6	
Potamopyrgus antipodarum	3.2	
Tubifex costatus	4.8	
Pygospio elegans	5.1	5.8
Macoma balthica	5.4	5.8
Hydrobia ulvae	5.6	2.6
Mya arenaria	5.7	5.8
Scoloplos armiger	6.1	7.2
Abra alba	7.0	4.0
Diastylis rathkei	7.1	8.8
Arctica islandica	7.6	7.6
Polydora quadrilobata	8.0	
Nephtys caeca	8.4	5.0
Spio filicornis	8.4	13.1
Mysella bidentata	9.1	7.5
Astarte borealis	10.0	
Parvicardium ovale	10.3	10.5
Corbula gibba	10.3	4.7
Ophiura albida	11.1	9.4
Astarte montagui	12.0	9.6
Molgula manhattensis	12.5	
Phoxocephalus holbolli	12.9	
Astarte elliptica	13.1	
Dendrodoa grossularia	13.8	
Edwardsia danica	14.1	13.7
Buccinum undatum	14.5	7.0
Macoma calcarea	15.6	9.2
Laonome kroeyeri	15.6	8.1
Ciona intestinalis	17.8	

[a] The values from the Kattegat/Skagerrak area are provided by www.marine-monitoring.se.

of the sampling locations ($n = 330$) were classified as "Moderate". A "High" EcoQ ($n = 42$) was observed only in areas with a higher salinity (>10 psu) whereas the EcoQ class "Bad" ($n = 34$) only occurred in areas with salinities below 10 psu. The distribution map reflects this pattern very well. With decreasing salinity from West to East (Fig. 2) or in direction to inner coastal waters the Shannon–Wiener index decreased as well. The most biodiverse stations were situated at the entrance of the Great Belt off the Island of Fehmarn. The EcoQ value "Good" was observed along the entire German coast with a clear clustering towards the West.

3.2. Benthic Quality Index (BQI)

The frequency distribution of the BQI and its variability in the southern Baltic Sea are presented in Fig. 5. The values ranged between 0.67 and 24.49. In contrast to the Shannon–Wiener index the EcoQ ($n = 405$) status observed most was in the category "Poor". About 13% ($n = 84$) of the sampling occasions were categorised as "Bad". Moreover, the BQI was strongly correlated to salinity. Almost all stations (307 out of 309) with salinities below 10 psu were classified as "Bad" or Poor" whereas localities with a salinity >10 psu tended to be categorised with a higher EcoQ. Due to the strong salinity gradient from West to East the distribution of EcoQ values "Good" and "High" was limited to the outermost West (in front of the Island of Fehmarn). East of the Island of Rugia only the category "Bad" and "Poor" were computed.

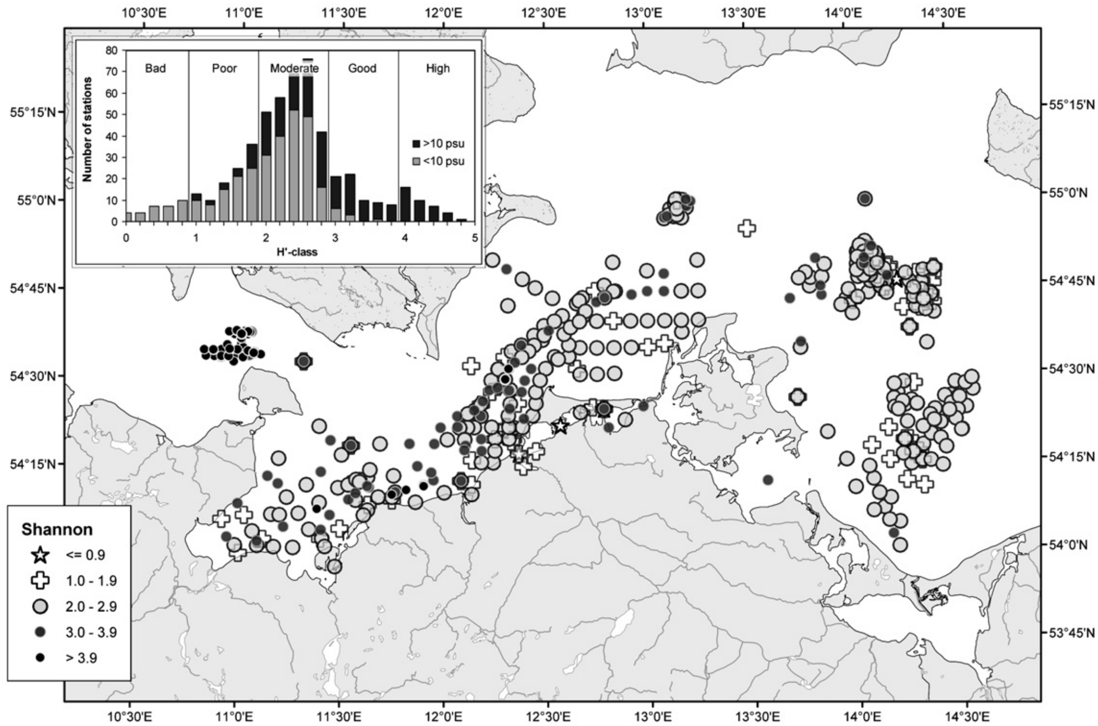

Fig. 4. EcoQ status and frequency distribution (histogram) of macrozoobenthos based on the Shannon–Wiener Index in the southern Baltic Sea. For the analysis 625 sampling occasions (period 1995–2005) were computed. The gray and black bars in the histogram refer to the salinity ranges of < and >10 psu, respectively.

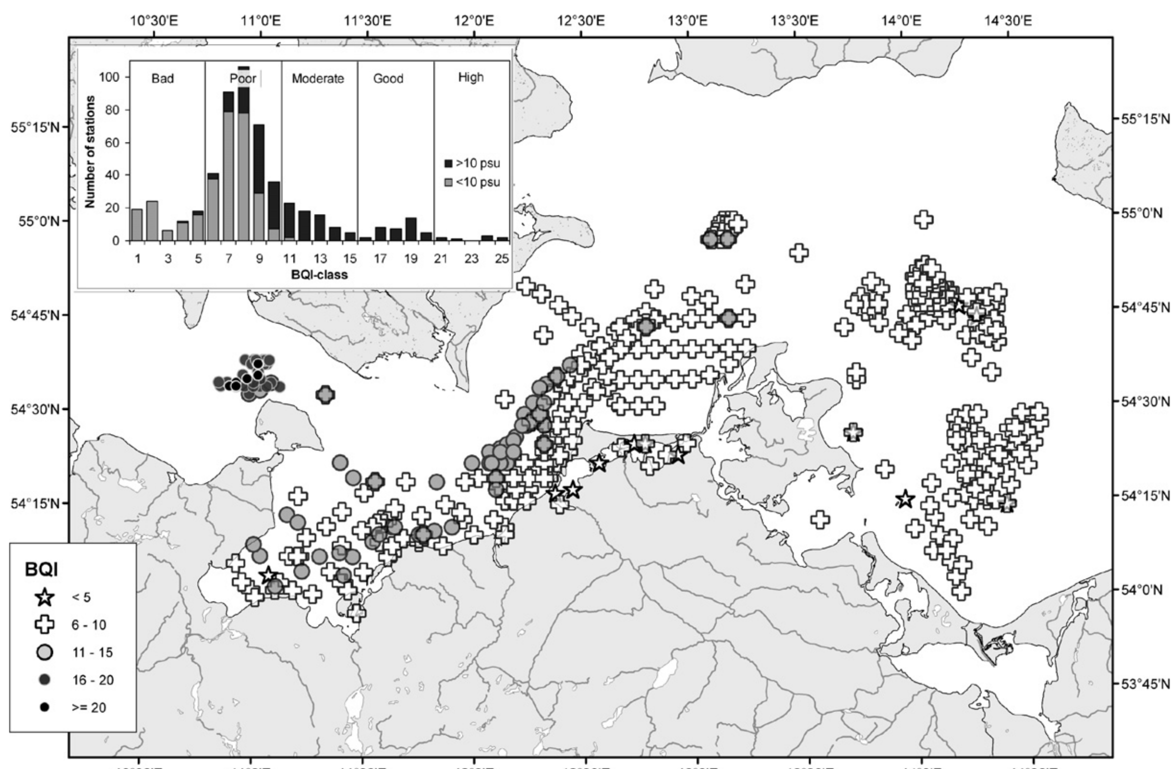

Fig. 5. EcoQ status and frequency distribution (histogram) of macrozoobenthos based on the BQI in the southern Baltic Sea. For the analysis 625 sampling occasions (period 1995–2005) were computed. The gray and black bars in the histogram refer to the salinity ranges of < and >10 psu, respectively.

3.3. Azti Marine Biotic Index (AMBI)

In opposite to the two biotic indices presented before when using the AMBI the calculated EcoQ was peaked in the category "Good" (Fig. 6). More than 70% of the stations distributed over the whole investigation area and thus salinity range belong to this class. The AMBI values ranged between 0.34 and 5.53. The 5 sampling occasions defined as "Poor" or "Bad" were characterised by a high dominance of oligochaete species or in the case of inner coastal waters by the polychaete *M. neglecta* and chironomids. No clear relation between AMBI and salinity gradient could be observed.

3.4. Comparison between indices and salinity

During the time period of the present study, the measured bottom water salinity ranged from 1.5 to 27.8 psu. The species number found per sampling occasion varied between 2 and 112 (Fig. 7a). Although low species numbers were observed over the whole salinity range a trend was obvious. With increasing salinity species number increased in a similar way. The maximum species number found at locations with salinities < and >10 psu was 53 (mean 24) and 112 (mean 31), respectively. The Shannon–Wiener index seems to be correlated with the species number (Figs. 7b and 8) since it increased with increasing salinity. The highest values (>4) and thus an EcoQ status of "High" were found only at salinities >10 psu. On the other hand, the macrozoobenthic communities in areas with salinities >10 were never computed as "Bad". However, all other categories were present in areas with salinities < and > 10 psu.

With regard to species number the BQI showed the strongest correlation to salinity (Figs. 7c and 8). All benthic communities in areas with a salinity <10 psu were categorised as "Bad" or "Poor". The EcoQ status "Moderate", "Good" or "High" and BQI values higher than 5 (except for three locations) were only given in areas with higher salinities. In contrast to the two indices presented above the AMBI showed only a weak correlation to salinity (Figs. 7d and 8). The mean AMBI for all sampling occasions was 2.49 in areas <10 psu and 2.46 in those >10 psu. In terms of EcoQ the majority of the benthic communities were defined as "Good". The sudden reduction in the EcoQ status of more or less two categories at salinities <7 psu is striking. About 73% of these stations were dominated by the genuine brackish water polychaete *M. neglecta*, oligochaetes and/or chironomids. Particularly in the brackish inner coastal waters and oligohaline waters east of the Island of Rugia these taxa found optimal living conditions.

3.5. Comparison between indices

Taking into account all sampling occasions and all three biotic indices, the EcoQ status represented by the macrozoobenthic communities covered all five categories as indicated in the Water Framework Directive. Nevertheless,

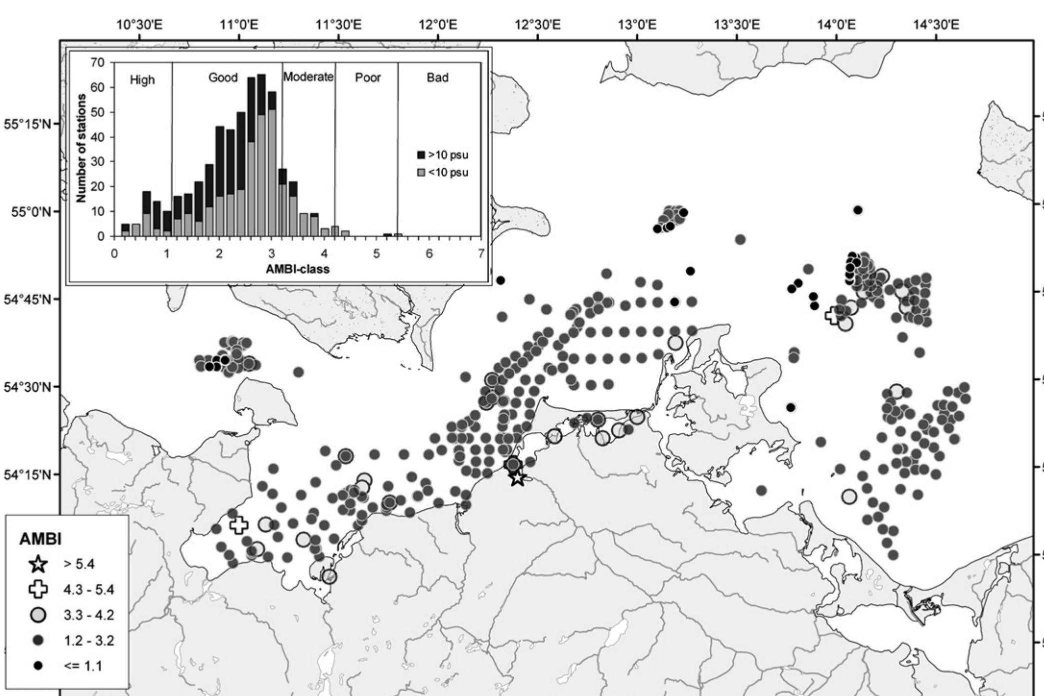

Fig. 6. EcoQ status and frequency distribution (histogram) of macrozoobenthos based on the AMBI in the southern Baltic Sea. For the analysis 625 sampling occasions between 1995 and 2005 were computed. The gray and black bars in the histogram refer to the salinity ranges of < and >10 psu, respectively.

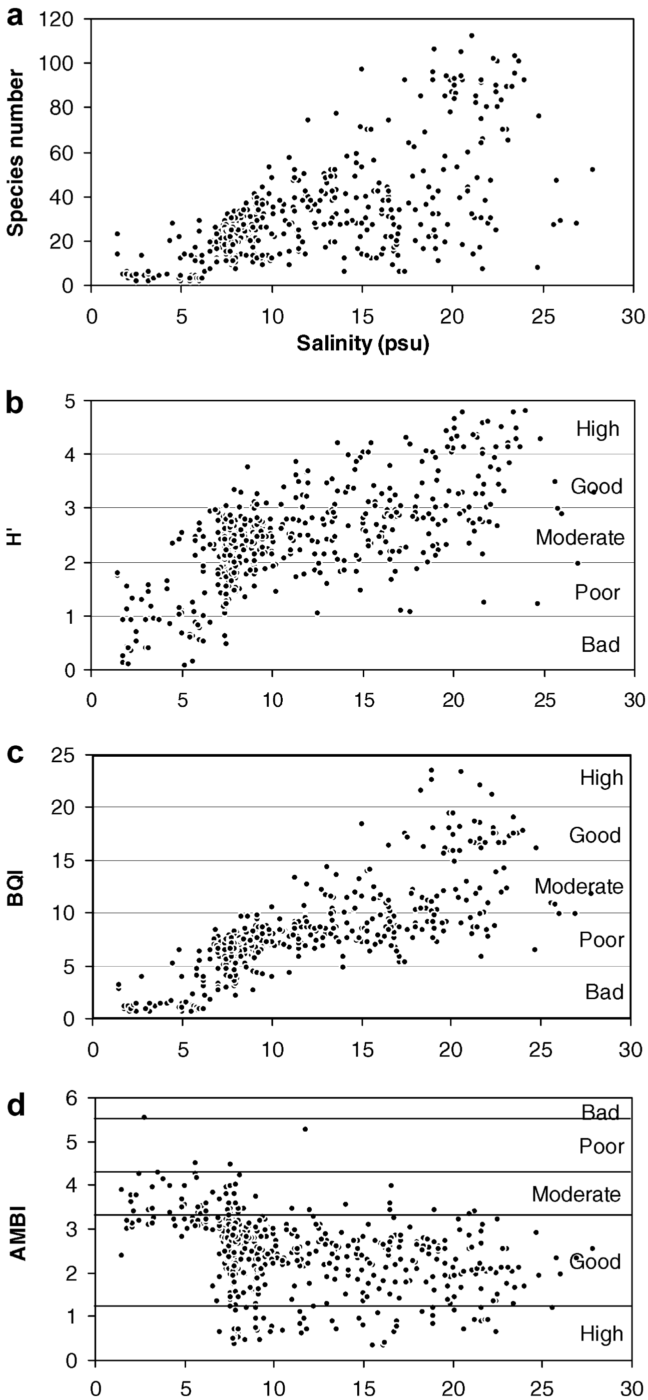

Fig. 7. Relationships between salinity and species number and the three biotic indices compared in the present study. The EcoQ status is indicated by the categories.

depending on the biotic index chosen the patterns were different and thus the overall assessment of the EcoQ status (Fig. 9). The quality status calculated by the BQI reached its maximum in the category "Poor" whereas the EcoQ status when using the AMBI was mainly "Good". The Shannon–Wiener index was more balanced and showed values in between the two others. The most significant correlation was given between H′ and BQI (Figs. 8 and 10a). The correlations between AMBI and H′ and BQI were weak but still statistically significant and negative (Figs. 8 and 10b, c) due to the opposite use of scaling (AMBI: the higher the value the lower the EcoQ; H′ and BQI: the higher the value the better the EcoQ).

All three relationships presented in Fig. 10 underline that the assessment of the EcoQ status for a given location depends on the biotic index applied. Only for a limited number of stations the same status was observed (Fig. 10a–c, grey areas). In 161 of 625 cases the relation between the EcoQ calculated by H′ and BQI were similar and lower (121) when comparing H′ and AMBI and lowest (45 cases) for AMBI and BQI.

3.6. Temporal changes in species numbers in the southern Baltic Sea

In Fig. 11a annual variations in species' numbers are shown for three monitoring stations. Owing to the different salinity regimes at the three stations (010 – mean bottom water salinity 24 psu, 030–14 psu, and 152–9 psu) species diversity differed clearly (Fig. 11a). The mean species number found during the last 10 years were 42 (station 010), 36 (030) and 21 (152), respectively.

The most dominant species at station 010 were the bivalves *Arctica islandica* and *Abra alba*, the polychaete *Lagis koreni* and the brittle star *Ophiura albida*. Station 030 was dominated by the gastropod *Hydrobia ulvae*, the bivalves *A. borealis* and *M. arenaria* and the polychaete *Scoloplos armiger*. Dominant species at the easternmost station 152 were the bivalves *M. balthica* and *Mytilus edulis* and the polychaete *P. elegans.*The found differences in the species numbers are also reflected by the biotic indices H′ and BQI (Fig. 11b and c) and the EcoQ status which ranged between "Poor" and "Good" and "Poor" and "Moderate", respectively. The AMBI value ranged between 1.4 and 3.1 and in respect to the EcoQ always indicated a "Good" status (Fig. 11d).

The macrofauna communities present at the monitoring stations not only depend on the salinity regime but on the occurrence and duration of oxygen depression periods. In the past 10 years only the westernmost station (010) was influenced by oxygen depletion (in 2002 and 2005) for several months. The oxygen depression was initiated in the Kiel Bight and reached the centre of the Mecklenburg Bight.

Until 2001 we observed a continuous increase in species' number at station 010. Due to the severe oxygen depletion in summer 2002 (and to a smaller extent in 2005) most of the benthic organisms became temporally extinct. Only the ocean quahog (*A. islandica*) and few miscellaneous species survived. The other two stations were not impacted by these events. Both the Shannon–Wiener index and the BQI reflect the collapse of the benthic community in a similar way. The H′ changed from "Good" to "Poor" (2 status classes in 2002 anyway) in terms of EcoQ and the BQI degraded 1 class from "Moderate" to "Poor". The AMBI

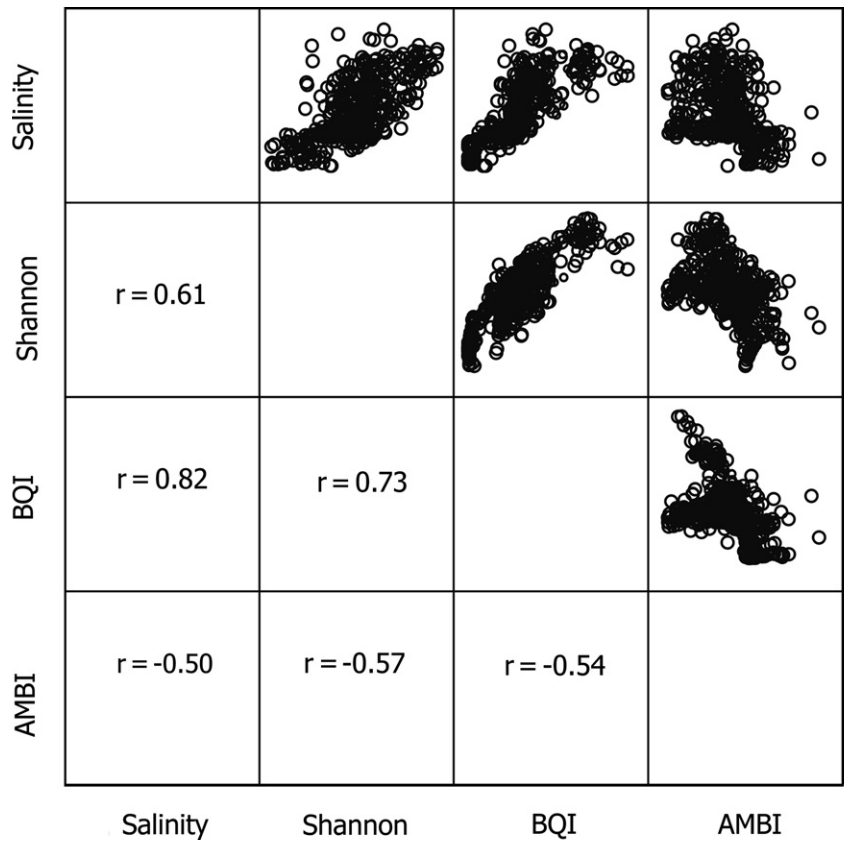

Fig. 8. Spearman–Rho correlation matrix and matrix scatter plot. All coefficients are significant at a level of $p < 0.01$. The number of pairs varies between 538 and 625.

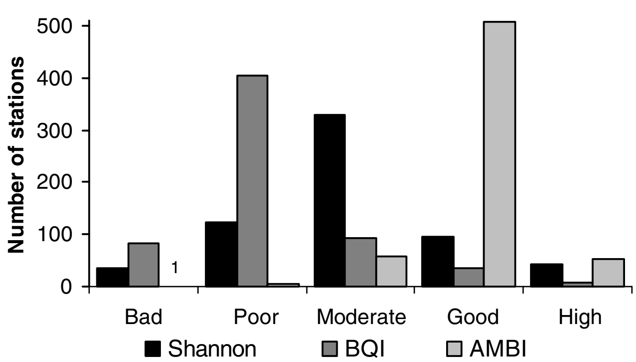

Fig. 9. Frequency distribution of EcoQ status of the 625 sites sampled between 1995 and 2005. Categorisation is based on Shannon–Wiener index, BQI and AMBI.

value, however, showed only a weak increase in 2002 and 2005 and in terms of EcoQ status no change was observed.

4. Discussion

4.1. Comparison between indices and salinity

In the present study, we have shown significant positive correlations between species number, H′, BQI and salinity which were strongest for the BQI resulting in a decreased biotic index with decreasing salinities. Therefore, a high ecological quality (EcoQ) as requested by the European Water Framework Directive will never be observed in brackish systems such as the Baltic with strong salinity gradients, particularly in the transition zone and in areas with low salinities. The salinity gradient acts as a natural stressor affecting benthic diversity in a similar way as human impact. Even in areas where the potential maximum species diversity will be reached, the H′ and BQI usually showed lower values than in more saline coastal waters. The EcoQ status of habitats with salinities <10 psu was always (with few exceptions) "Bad", "Poor" or "Moderate". The positive relationship between these two indices and salinity (with increasing salinity the species number increased as well) is not surprising since both of these indices account for species richness and dominance. An important difference between these two indices is the incorporation of the tolerance/sensitivity level of species when calculating the BQI and the $ES50_{0.05}$, respectively (Rosenberg et al., 2004). The BQI is based on individual datasets and the definition of "own" (regional) reference values. The calculation of the BQI in different marine areas is based on the calculation of $ES50_{0.05}$ for all species considered and on the computation of a reference value which is likely to be different depending on the habitat and benthic community present. For water depths lower and greater 20 m in the

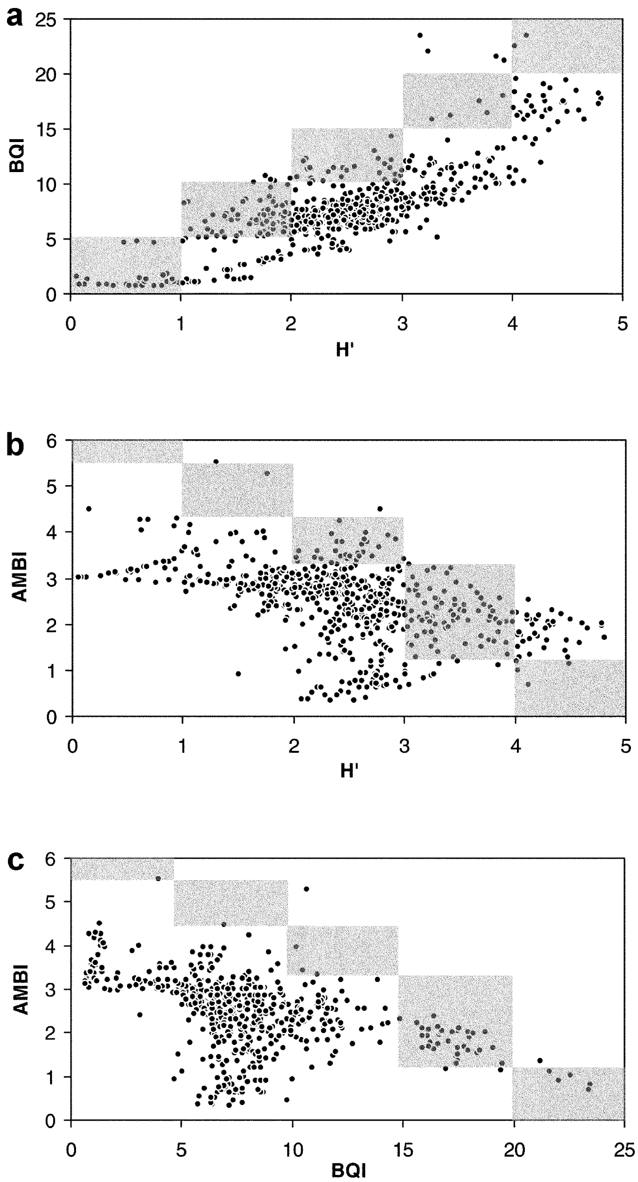

Fig. 10. Relationships between the three biotic indices compared in the present study. The grey areas indicate the overlapping EcoQ status calculated for each of the biotic indices. Only for the sampling occasions (dots) within these areas the assessment in terms of EcoQ were the same.

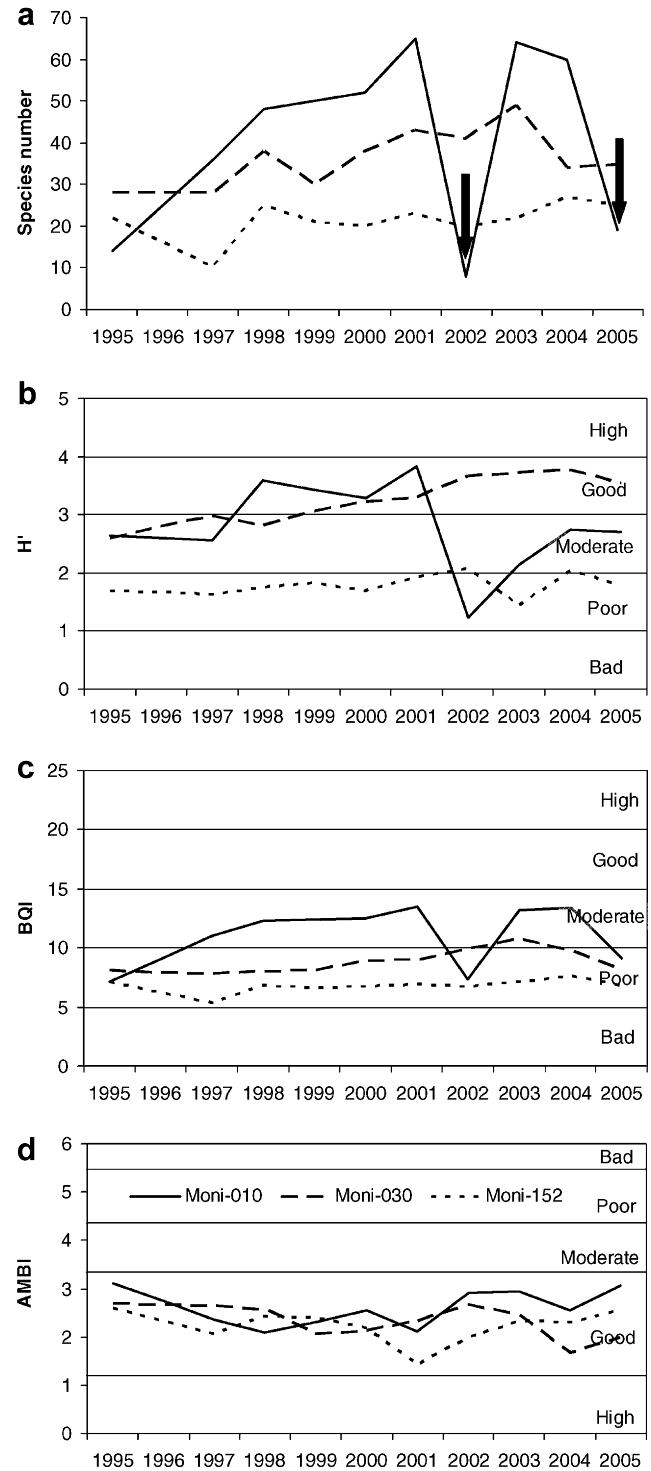

Fig. 11. Temporal changes in species number (a), H′ (b), BQI (c) and AMBI (d) at three selected monitoring sites (010, 030 and 152) in the southern Baltic Sea between 1995 and 2005. The mean bottom water salinities at the stations were 24, 14 and 9 psu, respectively. The arrows indicate oxygen depression periods in 2002 and 2005 at station 010.

Gulf of Lion, Labrune et al. (2006) calculated a maximum BQI (reference value) of 24.8 and 33.1, respectively. In the Skagerrak/Kattegat area Rosenberg et al. (2004) identified reference values of 18 and 20. In Reiss and Kröncke (2005) the maximum value for three locations in the North Sea was about 16. In the present study, the reference value for the southern Baltic Sea was 25, however, we did not distinguish between different water depths. In respect to salinity the reference values were 10 (<10 psu) and 25 (>10 psu), respectively. This implies a complete different categorisation in terms of EcoQ. This does not cause any problems when defining reference values for marine and brackish areas if they are separated a priori. In transition areas (like the southern Baltic) with strong salinity gradients and decreasing species numbers a different approach is probably needed by defining areas with specific salinity ranges (maybe with overlapping limits) and calculating reference values for each of them. The need to split and assess datasets relative to salinity following the umbrella WFD typology for the Baltic Sea as presented by Schernewski and Wilegat (2004) would be useful for implementation of the BQI in these coastal waters, resulting in a modified and less salinity biased classification. The use of the Venice system as proposed by Bald et al. (2005) might be another approach but probably not suitable for the southern Baltic Sea. Most of the stations presented here (about 80%) would be characterised as mesohaline (5–18 psu). As our results demonstrate differentiation of the benthic community due to salinity ranges and variability requires further subdivision of the region. In this respect, the umbrella typology mentioned above appears to be more appropriate.

When using the AMBI the EcoQ status of the macrozoobenthic communities in the southern Baltic Sea were categorised as "Good" over a wide regional range and relatively independent from the salinity. Both in areas with higher and lower salinities the most detected EcoQ status was "Good". The AMBI classification is mainly based on literature data regarding organic enrichment (Borja et al., 2000). For the AMBI, the tolerance/sensitivity level of species is assessed using a classification of five ecological groups (I–V). Within a group each species has been classified according to its reported tolerance/sensitivity to an environmental stress gradient. This classification is mainly based on published data or the experience of the authors (Grall and Glémarec, 1997; Borja et al., 2000; Borja et al., 2003; Muxika et al., 2005). The assigned ecological groups are discrete and supposed to be valid for all European areas at least and should not be biased by subjectivity. Our results show a somewhat different picture. The AMBI values obtained mainly ranged between 1.2 to 3.3 indicating a "good" status (Table 1) and a dominance of tolerance/sensitivity of species assigned to Group III (see also Borja and Muxika, 2005). For the AMBI the species number is not important but the ecological group the species belongs to (in most cases III) and its abundance. Borja and Muxika (2005) and Muxika et al. (2005) have stressed the limitations the AMBI may have in naturally-stressed and poor communities, e.g., inner parts of estuaries. The salinity gradient in the southern Baltic Sea probably causes similar restrictions when computing the AMBI. Borja and Muxika (2005) recommended in these particular cases to change the boundaries of the disturbance level (or comparing the results with reference conditions for these areas) rather than to modify the species ecological group assignations. When applying the AMBI another aspect has to be taken into account, i.e., the variability and changes in tolerance or sensitivity of species during their life cycle. The larvae or juveniles of species could have other sensitivities than the adults as pointed out by Rosenberg et al. (2004) for the polychaete *Capitella capitata* and the ocean quahog *A. islandica*.

4.2. Relationships between H′, BQI and AMBI

One main result of our study are the found distinct differences in the assessment of the ecological quality of macrozoobenthic communities in the southern Baltic Sea when using the three biotic indices. The discrepancy could range between 1 and 4 EcoQ status levels and only a small percentage of overlap was observed among the three indices. Similar to the results of Labrune et al. (2006) the best significant positive correlation was given between H′ and BQI, which is not surprisingly since both indices account for species richness and dominance. Opposite to Labrune et al. (2006) and to some extent Salas et al. (2004) the correlation of both H′ and AMBI and BQI and AMBI was negative (even though weak) as to be expected when considering the opposite use of scaling.

The different mode of including (or not in H′) and assessing (BQI and AMBI) tolerance/sensitivity level of species may have caused the significant differences between the biotic indices, however, the trend in assessing the EcoQ was very similar (Fig. 10). The EcoQ status was already lower due to the salinity reduction irrespectively of any anthropogenic impacts which may exist in this area.

The limitation of the assessment by biotic indices (particularly when based on benthic communities) appears to be due to a general overestimation of the species number on the one hand and overestimation of dominant species on the other hand. Moreover, these effects are contradictory when dominant species are categorised as disturbance-sensitive (AMBI) or have a wide salinity range of distribution and thus a low $ES50_{0.05}$ (BQI). In areas of salinity gradients like in the southern Baltic Sea it might be a better approach to (i) work with potentially (expected) species inventory and the real (attained) species; (ii) set salinity limits of BQI computation in respect to the typology of the WFD (see above); (iii) adapt the categorisation of tolerance/sensitivity level of species in AMBI and (iv) reduce the importance of dominant species. The first and the last point seem to be the most difficult ones. The potential species number of a subarea will only be obtained if information for the sampled habitat is available, a large data set for this type of habitat and for the investigation area and a representative time period. In principle this is similar to the knowledge of reference conditions (i.e., pristine areas). In trying to reconstruct the structure of benthic assemblages in the past, historic data (at least 100 years of information) are of great value (Zettler and Röhner, 2004). The overestimation of dominant species has to be considered, e.g., by data transformation. On the other hand the ranking of species in terms of their abundance has to be included.

The most important issue within the WFD is the comparison of data against reference conditions (Borja et al., 2004a,b; Borja et al., 2006; Muxika et al., 2006, this issue). The different methods presented by Borja et al. (2006, this issue) are potential approaches to fulfil the WFD requirement to determine reference conditions for each of the typologies and, likewise, to assess the EcoQ for each of the water bodies (including the determination of the boundaries). The main aim of the present paper was to analyse a comprehensive data set and to compare different established methods for assessing the ecological quality of waters in respect to macrozoobenthic communities. The definition of reference areas in the Southern Baltic Sea would be a follow up step towards an overall assessment of the ecological status in these coastal waters.

4.3. Temporal changes in biodiversity

In order to evaluated the natural annual variability of the three indices long term data sets (1995–2005) were used obtained at three monitoring stations covering a wide salinity range. To our knowledge no specific anthropogenic impacts (except for eutrophication in general) emerged during this period. However, at one of the station severe oxygen depression periods occurred during summer and autumn (2002 and 2005) in the past 10 years. As already pointed out, in terms of EcoQ the indices differed extremely (up to 3 classes) for the same sampling location. Apart from that all three indices seem to be less influenced by natural variability of the macrofauna communities. Particularly the results for the AMBI coincidence with the findings of Salas et al. (2004) and Reiss and Kröncke (2005) who showed that the AMBI varies only slightly in time. In the present study all three stations (although with salinity differences between 9 and 24 psu) were characterised as "Good" in terms of EcoQ. However, the strong impact of the oxygen depression period was only reflected by H′ and BQI. The AMBI did not respond to these drastic changes in the benthic community. Even though only very few species (e.g., *A. islandica*) of a macrozoobenthic community comprising originally of more than 60 species did survive this severe oxygen depression period, the EcoQ still indicated the category "Good" (Fig. 11d). This is a clear indication that not one single index should be used when assessing the EcoQ as also pointed out by Borja and Muxika (2005). In order to overcome this limitation, Muxika et al., this issue, 2006 have developed the Multivariate-AMBI, a combination of AMBI, species richness and Shannon Wiener index. In a future study it has to be tested if such an approach of an area specific classification of tolerance/sensitivity levels is practicable in areas with temporal low oxygen concentrations. Many of the high ecological assigned species (e.g., the bivalves *Astarte* spp., *M. balthica*) are tolerant to short term (weeks) hypoxia events by closing their valves, such behaviour was not detected by the AMBI. This is different in inner coastal areas with longer lasting oxygen depletion due to pollution resulting in a very impoverished community or even azoic conditions as found in the Nervion estuary in the southern Bay of Biscay. In this case the AMBI was significantly related to oxygen availability (Borja et al., 2006). The need to adjust this biotic index is also given due to the replacement of *C. capitata* in disturbed marine areas by the bivalve *M. balthica* and by oligochaetes in the low salinity areas of the Northern Baltic (Pearson and Rosenberg, 1978; Rosenberg et al., 2004).

5. Conclusions

Our study clearly demonstrates that in areas with strong salinity gradients such as the southern Baltic Sea the EcoQ classification based on macrozoobenthic communities as indicator greatly depends on the biotic index chosen. This is mainly caused by (i) the dependency on species richness, (ii) the over-estimation of dominant species and (iii) the different categorisation of tolerance/sensitivity level of species. The lack of considering species indication by H′ may make this index inappropriate to compare areas featuring different biodiversity levels. The greatest advantage of the AMBI, the discrete species list with its categorisation from very sensitive (Group I) to first-order opportunistic (Group V) species, seems to be a disadvantage in this gradient system. Particularly when dominant species are not classified accordingly it may result in inappropriate assessment of the EcoQ. Modified indices like the M-AMBI (Muxika et al., 2006, this issue) in combination with the salinity typology (Bald et al., 2005) could be an appropriate way to assess the EcoQ in waters with a strong salinity gradient. The BQI seems to be suitable for areas with strong salinity gradients but only when including tolerance/sensitivity level of species based on individual data sets and of data-specific (or area-specific) reference values. Furthermore, it should be adjusted to the simple umbrella typology for the Baltic Sea according to the WFD. The species specific $ES50_{0.05}$ values and the reference values should be calculated for discrete salinity ranges (taking into account water retention time and depth). The danger of overestimating dominant species has to be considered. Another approach could be to combine all three biotic indices. The Multivariate-AMBI (including species richness, Shannon Wiener index and AMBI) is one step in this direction (Muxika et al., 2006, this issue). The intercalibration of methods from Spain, Denmark, United Kingdom and Norway is another important approach to compare and adapt the indices (Borja et al., 2006, this issue). Presently no biotic index seems to be adjusted for application in the southern Baltic Sea with its salinity gradient. The need of an user-friendly biotic index to fulfil the WFD and the risk for a drastic reduction of the initial environmental information when using a single biotic index still needs further consideration.

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References

Bald, J., Borja, A., Muxika, I., Franco, J., Valencia, V., 2005. Assessing reference conditions and physico-chemical status according to the European water framework directive: a case-study from the Basque country (Northern Spain). Marine Pollution Bulletin 50, 1508–1522.

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 333, 383–391.

Borja, A., Muxika, I., 2005. Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality. Marine Pollution Bulletin 50, 787–789.

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.

Borja, A., Muxika, I., Franco, J., 2003. The application of a Marine Biotic Index to different impact source affecting soft-bottom benthic communities along European coasts. Marine Pollution Bulletin 46, 835–845.

Borja, A., Franco, J., Muxika, I., 2004a. The biotic indices and the Water Framework Directive: the required consensus in the new benthic monitoring tools. Marine Pollution Bulletin 48, 405–408.

Borja, A., Franco, J., Valencia, V., Bald, J., Muxika, I., Belzunce, M.J., Solaun, O., 2004b. Implementation of the European Water Framework Directive from the Basque country (Northern Spain): a methodological approach. Marine Pollution Bulletin 48, 209–218.

Borja, A., Josefson, A.B., Miles, A., Muxika, I., Olsgard, F., Phillips, G., Rodriguez, J.G., 2006. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. Marine Pollution Bulletin, this issue, doi:10.1016/j.marpolbul.2006.08.018.

Borja, A., Muxika, I., Franco, J., 2006. Long-term recovery of soft-bottom benthos following urban and industrial sewage treatment in the Nervion estuary (southern Bay of Biscay). Marine Ecology Progress Series 313, 43–55.

Clarke, K.R., Warwick, R.M., 1994. Change in marine communities: an approach to statistical analysis and interpretation. Plymouth Marine Laboratory.

Costello, M.K., Emblow, C.S., White, R., 2001. European register of marine species. A check-list of the marine species in Europe and a bibliography of guides to their identification. Patrimoines Naturels 50, 1–463.

Grall, J., Glémarec, M., 1997. Using biotic indices to estimate macrobenthic community pertubations in the Bay of Brest. Estuarine, Coastal and Shelf Science 44 (Suppl. A), 43–53.

Jansson, B.-O., Jansson, A.M., 2002. The Baltic Sea: reversible unstable or irreversible stable? In: Gundersen, L.H., Pritchard, L.P. (Eds.), Resilience and Behaviour of Large-Scale Ecosystems. Island Press, Washington DC, pp. 71–108.

Labrune, C., Amouroux, J.M., Sarda, R., Dutrieux, E., Thorin, S., Rosenberg, R., Grémare, 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.

Muxika, I., Borja, A., Bonne, W., 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecological Indicators 5, 19–31.

Muxika, I., Borja, A., Bald, J., 2006. 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, this issue, doi:10.1016/j.marpolbul.2006.05.025.

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the amrine environment. Oceanography and Marine Biology: An Annual Review 16, 229–311.

Perus, J., Bäck, S., Lax, H.-G., Westberg, V., Kauppila, P., Bonsdorff, E., 2004. Coastal marine zoobenthos as an ecological quality element: a test of environmental typology and Europeaan Water Framework Directive. Coastline Reports 4, 27–38.

Pielou, E.C., 1975. Ecological Diversity. John Wiley and Sons, New York, pp. 165.

Reiss, H., Kröncke, I., 2005. Seasonal variability of benthic indices: an approach to test the applicability of different indices for ecosystem quality assessment. Marine Pollution Bulletin 50, 1490–1499.

Rönnberg, C., Bonsdorff, E., 2004. Baltic Sea eutrophication: area-specific ecological consequences. Hydrobiologia 514, 227–241.

Rosenberg, R., Blomquist, 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.

Rumohr, H., Bonsdorff, E., Pearson, T.H., 1996. Zoobenthic succession in Baltic sedimentary habitats. Archive of Fishery and Marine Research 44, 179–214.

Salas, F., Neto, J.M., Borja, A., Marques, J.C., 2004. Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems: the case of Mondego estuary (Portugal). Ecological Indicators 4, 215–225.

Schernewski, G., Wilegat, M., 2004. Towards a typology for the Baltic Sea. Coastline Reports 2, 35–52.

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, 77–111.

Vincent, C., Heinrich, H., Edwards, A., Nygaard, K., Hayhornthwaite, J., 2002. Guidance on typology, reference conditions and classification systems for transitional and coastal waters. CIS Working Group 2.4 (COAST), Common Implementation Strategy of the Water Framework Directive, European Commission, pp. 119.

Zettler, M.L., Röhner, M., 2004. Verbreitung und Entwicklung des Makrozoobenthos der Ostsee zwischen Fehmarnbelt und Usedom - Daten von 1839 bis 2001. In: Bundesanstalt für Gewässerkunde (Hrsg.), Die Biodiversität in Nord- und Ostsee, Band 3. Bericht BfG-1421, Koblenz, pp. 175.

Zettler, M.L., Daunys, D., Kotta, J., Bick, A., 2002. History and success of an invasion into the Baltic Sea: the polychaete *Marenzelleria* cf. *viridis*, development and strategies. In: Leppäkoski, E., Olenin, S., Gollasch, S. (Eds.), Invasive Aquatic Species of Europe, Distribution, Impacts and Management. Kluwer Academic Publishers, Dordrecht, pp. 66–75.