

KLAIPĖDA UNIVERSITY

Romualda CHUŠEVĖ

EVALUATION OF MACROZOOBENTHOS SPECIES  
SENSITIVITY AND APPLICATION OF BENTHIC  
QUALITY INDEX FOR THE SEABED STATUS  
ASSESSMENT OF THE SOUTH-EASTERN BALTIC SEA

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VERTINIMAS IR BENTOSO KOKYBĖS INDEKSO  
TAIKYMAS VERTINANT PIETRYTINĖS BALTIJOS  
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# **Abstract**

This study provides the analysis of the macrozoobenthos species sensitivity values and application of Benthic Quality Index (BQI) for the assessment of the south-eastern part of the Baltic Sea. The numerical sensitivity concept and its use in BQI were tested for different depths, macrozoobenthos communities and types of anthropogenic disturbance. The BQI was validated against total nitrogen, total phosphorus and chlorophyll concentrations, and the dynamics of macrozoobenthos sensitivity groups was used to characterise water quality class boundaries for the coastal waters. The specificity and sensitivity of the BQI was assessed using the non-parametric Signal detection theory method and its performance in relation to eutrophication parameters was tested for the open coastal waters and plume of the Curonian Lagoon. Additionally, the detailed recommendations for the coastal water status assessment in the south-eastern part of the Baltic Sea were provided.

## **Key words**

Benthic macroinvertebrates, coastal water status assessment, human impact, index specificity, Signal detection theory method, threshold determination

## Reziumė

Disertacijoje pateikiama makrozoobentos rūšių jautrumo analizė ir bentoso kokybės indekso (BQI) panaudojimas vertinant pietrytinę Baltijos jūros dalį. Marozoobentos jautrumo ir BQI indekso vertės buvo analizuotos skirtingiems gyliams, makrozoobentos bendrijoms ir antropogeniniams poveikiams. Taip pat indekso vertės buvo validuotos su bendro azoto, fosforo ir chlorofilo-a koncentracijomis priekrantėje. Pagal bentoso kokybės indekso ryšį su rūšių jautrumo grupių gausumo pasiskirstymu buvo nustatytos vandens būklės klasės priekrantėje. Panaudojant Signalo aptikimo teorijos metodą įvertintas indekso jautrumas ir specifiškumas, bei jo atsakas į eutrofikacijos procesą apibūdinančius parametrus priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje. Pateiktos detalios rekomendacijos kaip vertinti vandens būklę bei nustatyti jų slenkstines vertes naudojant makrozoobentos rūšių jautrumo vertes bei bentoso kokybės indeksą pietrytinėje Baltijos jūros dalyje.

## Reikšmingi žodžiai

Dugno bestuburiai, priekrantės vandenų būklės vertinimas, antropogeninis poveikis, indekso specifiškumas, Signalo aptikimo teorijos metodas, slenkstinių verčių nustatymas

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# 1

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## Introduction

### 1.1 Relevance of the thesis

Healthy marine environments with diverse biological communities and good environmental status provide valuable ecosystem services and support a wide range of human activities. The requirement to assess the environmental status of marine waters is growing across continents (Borja et al., 2008). In Europe, the umbrella regulations for addressing the ecological quality of aquatic systems are the WFD (2000/60/EC) for lakes, rivers, transitional (estuaries and lagoons) and coastal waters, and the MSFD (2008/56/EC) for marine waters.

It is widely accepted that macrozoobenthos species and communities reflect natural and anthropogenic changes in marine ecosystems, as they are unable to avoid unfavorable conditions, have a long reproductive cycle, accumulate changes over time and occur at various depths (Dauer, 1993; Borja et al., 2000; Zettler et al., 2007). Therefore structural parameters of bottom macrofauna communities were synthesized in a number of multimetric indices to be used for environmental status assessment 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 Polychaete and Amphipod Index (BOPA; Dauvin and Ruellet, 2007) and the Benthic Opportunistic Annelida Amphipoda Index (BO2A; Dauvin and Ruellet, 2009).

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Many authors agree that eutrophication, chemical pollution and mechanical disturbance of the sea bottom are the major anthropogenic pressures determining changes in macrozoobenthos abundance, distribution and species composition (McQuatters-Gollop et al., 2009; Van Hoey et al., 2010; Rice et al., 2012). In the face of these multiple pressures, an accurate assessment of environmental status is a prerequisite for establishing environmental targets and selecting specific management measures. The crucial step here is the selection of appropriate indicators, therefore this issue is addressed in many studies (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 among the selection criteria (Rombouts et al., 2013). Once an indicator has been developed, its performance in terms of sensitivity (i.e. the response to an existing disturbance), specificity (i.e. the resistance to the noise or non-targeted disturbance) and the accuracy in relation to the actual response can be evaluated (Murtaugh, 1996).

In spite of selection criteria, the performance of indicators is unlikely to be consistent across habitats and ecosystems, since bottom-dwelling organisms are not equally sensitive to different types of anthropogenic disturbances (Buhl-Mortensen et al., 2009), or environmental conditions (Tagliapietra et al., 2009). Following guidance for recent assessments of environmental status (WFD, 2000/60/EC; MSFD, 2008/56/EC; HELCOM, 2012), benthic indices should integrate sensitivity, richness and abundance of macrozoobenthos species. While richness and abundance are directly assessed from the benthic samples, defining species sensitivity is less straightforward.

Generally, sensitivity is described as a product of a likelihood of damage due to pressure (Laffoley et al., 2000; OSPAR, 2008). Typically, very sensitive species react fast to the impact of natural stressors or human activity and recover only after a prolonged period, if at all. Species grouping into sensitivity classes based on expert valuation is used in several indices (e.g. AMBI, Borja et al., 2000; BENTIX; Simboura and Zenetos, 2002; M-AMBI, Muxika et al., 2007, Leonardsson et al., 2009). Typically this approach enables one to take into account different sensitivity meanings (e.g. resilience, resistance, recoverability and vulnerability) (Tyler-Walters et al., 2001), which otherwise are difficult to quantify using numerical algorithms. Additionally, expert-based sensitivity values may have a fixed range of variability, predefined number of sensitivity classes and fixed distance between sensitivity values for organisms belonging to different sensitivity classes. These features make species sensitivity estimates well-structured and simple to interpret in a context of overall status assessment.

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In contrast to expert opinion, a standardized mathematical algorithm for assessing species sensitivity has an obvious advantage for screening sensitivity performance under different conditions. It also makes sensitivity values comparable between different areas and datasets leaving less freedom for low certainty assumptions. So far, the only standardized species sensitivity assessment algorithm was proposed by Rosenberg et al. (2004) and recently revised by Leonardsson et al. (2015). The overall concept relies on the assumption that lower sensitivity species will be more abundant and will occur more frequently in disturbed sites, characterized by lower species richness, while species of higher sensitivity will attain higher abundance at higher species richness i.e. less disturbed sites. This implies that sensitivity value and the overall assessment is dependent on the diversity range (i.e. disturbance gradient) covered by the dataset.

In this study the reliability of macrozoobenthos species sensitivity estimates and its influence on the index values were tested in a context of depth gradient, changing communities, type and level of anthropogenic disturbances where the BQI values respond to the analysed parameters significantly differently. Additionally, specificity and sensitivity of the BQI was assessed using the non-parametric Signal detection theory method and recommendations for the index applications are provided.

### 1.2 Aim and objectives

The aim of the study is to evaluate variability patterns in macrozoobenthos species sensitivity values and their role in applications of Benthic Quality Index for the ecosystem status assessment in low diversity communities of the south-eastern Baltic Sea.

The following objectives were raised for this work:

1. To assess the importance of a depth gradient for the species sensitivity values and their use in Benthic Quality Index.
2. To quantify the variability of the species sensitivity values and Benthic Quality Index under changing macrozoobenthos community structure.
3. To evaluate the reliability of the species sensitivity values in a context of different anthropogenic pressures and variable disturbance level.
4. To test the application of the Signal detection theory for the validation of Benthic Quality Index values and determination of water quality class thresholds.
5. To develop recommendations for the water quality status assessment of the south-eastern Baltic Sea using Benthic Quality Index.

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### **1.3 Novelty**

This study provides new results on changes of macrozoobenthos species sensitivity estimates for different disturbance types under changing environmental conditions and their influence for BQI variability. This study carried out on macrozoobenthos of the south-eastern Baltic Sea confirmed very recent results (Schiele et al., 2016; Leonardsson et al., 2016) of the dependency of species sensitivity estimates on the depth. However, this phenomenon was demonstrated for much smaller spatial scale and very narrow depth range and therefore, findings of this study have a new perspective and interpretation. The consistency analysis for macrozoobenthos species sensitivity values showed their considerable variation between two macrofauna communities within the same depth range and under similar sediment types, what has not been demonstrated during earlier studies and neglected by previous status assessments in the Baltic. This study also tested the application of the Signal detection theory for the validation of macrozoobenthos based quality index and setting up thresholds for quality classes. Although newly applied in a context of marine ecosystem status assessment, this method demonstrated relatively good results as noticed by Heiskanen et al. (2016). BQI was also exposed to a new assessment system consisting of eight quality criteria and this exercise delivered new knowledge in evaluation of the benthic indicator in a context of Biodiversity (D1) and Sea floor integrity (D6) Descriptors of the Marine Strategy Framework Directive (MSDF; European Commission 2008/56/EC).

### **1.4 Scientific and applied significance of the results**

The major scientific importance of the current study lies in advanced understanding of reliability and accuracy of the species sensitivity estimates gained from typical datasets delivered by environmental monitoring programmes and short-term research projects. The applied value of the study is built on the background knowledge and detailed guidelines for the assessment of the soft bottom status of the south-eastern Baltic Sea using BQI index. This knowledge and guidelines include index validation, estimated species sensitivity values and justified boundaries for water quality classes, which have been approved by the Lithuanian Ministry of Environment for the water quality assessment in the framework of Water Framework Directive and Marine Strategy Framework Directive (2000/60/EC; 2008/56/EC).

### **1.5 Defensive statements**

1. Macrozoobenthos species sensitivity values reflect both species response to natural variability and anthropogenic disturbance, therefore water quality assessment

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using Benthic Quality Index should be carried out for precisely defined setting of environmental conditions, benthic communities and acting disturbances.

2. The set of sensitive and tolerant species differ between types of anthropogenic disturbance, whereas species sensitivity values may depend on coverage of disturbance gradient by the dataset.

3. The Signal detection theory method can be applied for the Benthic Quality Index validation against selected disturbance parameters and setting the water quality class boundaries.

### 1.6 Scientific approval

Results of this study were presented in 3 international and 2 national conferences: ECSA 56 Coastal systems in transition: From a 'natural' to an 'anthropogenically-modified' state conference. Bremen, Germany, September, 2016.

Scientific symposium 2015: "Tools for assessing status of European aquatic ecosystems", Malme, Sweden, May 2015.

10th Baltic Sea Science Congress, Riga, Latvia, June, 2015.

9th national conference „Marine and coastal research“. Klaipėda, Lithuania, April, 2016.

10th national conference, „Marine and coastal research“. Palanga, Lithuania, April, 2017.

The material of this study was presented in 3 original publications, published in peer-reviewed scientific journals:

**Chuševė, R.**, Nygård, H., Vaičiūtė, D., Daunys, D., Zaiko A. (2016) Application of signal detection theory approach for setting thresholds in benthic quality assessments. *Ecological Indicators* 60, 420–427.

Queiros, A.M., Strong J. A, Mazik, K., Carstensen, J., Bruun, J., Somerfield, P. J., Bruhn, A., Ciavatta, S., **Chuševė R.**, Nygård, H., Flo E., Bizsel N., Ozaydinli, M., Muxika I., Papadopoulou, N., Pantazi, M., Krause-Jensen, D. (2016) An objective framework to test the quality of candidate indicators of good environmental status. *Frontiers in Marine Science*. 10.3389/fmars.2016.00073.

**Chuševė, R.**, Daunys, D. (2017) Can benthic quality assessment be impaired by uncertain species sensitivities? *Marine Pollution Bulletin*. 116, 332–339.

### 1.7 Thesis structure

The dissertation includes eighth chapters: introduction, literature review, material and methods, results, discussion, conclusions, references. The material is presented in 128 pages, 23 figures and 13 tables. The dissertation refers to 119 literature sources. Dissertation is written in English with an extended summary in Lithuanian language.

### 1.8 Acknowledgments

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Macrozoobenthos samples were collected in the framework of several projects: EIA project “The use of wind energy in the open waters of Šventosios-Palangos part” (2016), DENOFLIT project (Inventory of marine species and habitats for development of Natura 2000 network in the offshore waters of Lithuania) co-financed by European Union LIFE Programme and the Republic of Lithuania (Project No. LIFE09 NAT/LT/000234) and the project “A system for the sustainable management of Lithuanian marine resources using novel surveillance, modeling tools and an ecosystem approach” funded by Norwegian Financial Mechanism and the Republic of Lithuania. National monitoring data was provided by the Marine Research Department (Environmental Protection Agency, Lithuanian Ministry of Environment).

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### 1.9 Abbreviations

| Abbreviation  | Explanation                                             |
|---------------|---------------------------------------------------------|
| <b>AMBI</b>   | AZTI Marine Biotic Index                                |
| <b>ANOSIM</b> | Analysis of similarities (statistical term)             |
| <b>AUC</b>    | The area under the ROC curve (statistical term)         |
| <b>BQI</b>    | Benthic Quality Index                                   |
| <b>BBI</b>    | Brackish Water Benthic Index                            |
| <b>BENTIX</b> | Biotic Index                                            |
| <b>BOPA</b>   | Benthic Opportunistic Polychaeta Amphipoda Index        |
| <b>CDOM</b>   | Coloured dissolved organic matter                       |
| <b>CHL-a</b>  | Chlorophyll a concentration                             |
| <b>DKI</b>    | Danish multimetric quality index                        |
| <b>EEZ</b>    | Exclusive Economic Zone                                 |
| <b>EU</b>     | European Union                                          |
| <b>EG</b>     | Ecological group                                        |
| <b>HELCOM</b> | Helsinki Commission                                     |
| <b>ITI</b>    | Infaunal Trophic Index                                  |
| <b>LMA</b>    | Lithuanian marine area                                  |
| <b>MarBIT</b> | Marine Biotic Index Tool                                |
| <b>MERIS</b>  | The cloud free MEdium Resolution Imaging Spectrometer   |
| <b>MSFD</b>   | Marine Strategy Framework Directive (2008/56/EC)        |
| <b>M-AMBI</b> | Multivariate-AMBI                                       |
| <b>nMDS</b>   | Non-metric multidimensional scaling (statistical term)  |
| <b>NPV</b>    | Negative predictive value                               |
| <b>PPV</b>    | Positive predictive value                               |
| <b>ROC</b>    | Receiver Operating Characteristic (statistical term)    |
| <b>SDT</b>    | Signal detection theory                                 |
| <b>SIMPER</b> | Statistics of similarity percentages (statistical term) |
| <b>TN</b>     | Total nitrogen concentration                            |
| <b>TP</b>     | Total phosphorus concentration                          |
| <b>WFD</b>    | Water Framework Directive (2000/60/EC)                  |
| <b>ZKI</b>    | Macrozoobenthos community index                         |





# 2

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## Literature review

### 2.1 Review of the macrozoobenthos indexes

A variety of macrozoobenthos indicators have been developed during the last decade to assess the status of coastal and open waters using defined target values and reference conditions (Borja et al., 2009; Hering et al., 2010). Several characteristics make macrozoobenthos organisms useful and suitable indicators, as they

1. live in the bottom sediments, where exposure to contaminants and oxygen stress is most frequent (Kennish, 1992; Engle, 2000);
2. are relatively sedentary and reflect the quality of their immediate environment (Pearson and Rosenberg, 1978; Dauer, 1993);
3. have relatively long life span and species responses integrate water and sediment quality changes over time (Dauer, 1993; Reiss and Kroncke, 2005);
4. include diverse species with a variety of life features and tolerance levels to stress, which allow their inclusion into different functional response groups (Pearson and Rosenberg, 1978);
5. some species are commercially important species or their preys (Reiss and Kroncke, 2005);
6. affect fluxes of chemicals between sediment and water columns through bioturbation and suspension feeding activities, as well as playing a vital role in nutrient cycling (Reiss and Kroncke, 2005).

## 2. Literature review

Yet the performance of benthic 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), to geographical specifications (Dauvin, 2007) or environmental conditions (Tagliapietra et al., 2009). An ideal indicator should be responsive to any stressor type, have a low natural variability, provide a response that can be distinguished from natural variations and be interpretable (Hering et al., 2006). It has been shown that the main differences between the indicators can be attributed to the differences in (1) sensitivity, (2) susceptibility to natural variability, (3) types of included variables, (4) the method used to determine sensitivity of species, and (5) the reaction to the sampling strategy (Hoey et al., 2010).

A series of multimetric macrozoobenthos indices used for the seabed status assessment can be grouped into the three major categories based on:

- (i) diversity–Shannon-Weaver diversity index  $H'$  (Shannon, 1948), Benthic Quality Index (BQI) (Rosenberg et al., 2004);
- (ii) ecological groups–AMBI (AZTI Marine Biotic Index, Borja et al., 2000); M-AMBI (Multivariate-AMBI, Muxika et al., 2007); BENTIX (Biotic Index, Simboura and Zenetos, 2002); BOPA (Benthic Opportunistic Polychaeta Amphipoda Index, Dauvin and Ruellet, 2007);
- (iii) trophic groups–ITI (Infaunal Trophic Index; Mearns and Word, 1982).

### 2.1.1 Indices based on ecological groups

**AZTI Marine Biotic Index (Borja et al., 2000).** The AZTI Marine Biotic Index (AMBI) relies on the abundance distribution of the soft-bottom macrozoobenthos organisms, classified into five EG based on their sensitivity to organic enrichment: EG I being described as the disturbance-sensitive species, EG II-disturbance-indifferent species, EG III-disturbance-tolerant species, EG IV-second-order opportunistic species and EG V-first-order opportunistic species) (Hily, 1984; Glemarec, 1986; Grall and Glemarec, 1997). This index is calculated according to the weighted relative abundance of each EG in a site:

$$AMBI = \left[ \frac{(0 \times \%EGI) + (1.5 \times \%EGII) + (3 \times \%EGIII) + (4.5 \times \%EGIV) + (6 \times \%EGV)}{100} \right] \quad (1)$$

The index result is a continuous number varying between 0 (for unpolluted sites) and 7 (for extremely polluted sites). The index was tested and provided reliable results in geographically different sites: a number of European waters (Muxika et al., 2005) including coastal waters along the Basque Country, Spain (Borja et al., 2000), the Mondego estuary, Portugal (Salas et al., 2004), areas along the Brazilian coast and off

the Uruguayan coast (Muniz et al., 2005). It was proved that AMBI reacts in the same way to different disturbance sources, e.g. anoxic episodes, hydrocarbon pollution, engineering works, dredging or fish farming cages (Borja et al., 2000, 2003).

One disadvantage of AMBI is the potential uncertainties in the species ecological grouping. Once it draws on the response of organisms to organic inputs in the ecosystem it does not detect the effects caused by other types of pollution, as for instance toxic pollution (Marin-Guirao et al., 2005). Moreover, AMBI has not been shown to be useful for poor communities in naturally stressed environments, e.g. high hydrodynamic energy areas, subtidal sandbanks, and the inner parts of the estuaries (Muxika et al., 2005).

**Multivariate-AMBI (Muxika et al., 2007).** The Multivariate-AMBI (M-AMBI) is a combination of the proportion of ‘disturbance-sensitive taxa’ (through the computation of the AMBI index) and species diversity (through the use of the Shannon-Weaver diversity index  $H'$ ). These parameters are integrated through the use of discriminant analysis (DA) and factorial analysis (FA) techniques.

The M-AMBI has been the outcome of the intercalibration process among member states for the WFD (2000/60/EC). Nevertheless, it has been applied to other systems outside the Europe, e.g. Chesapeake Bay, USA, where it revealed to be a consistent measure, providing high agreement with local indices (Borja et al., 2008). The main advantage attributed to this index, as well as of AMBI, is that both are easily computed, and the software is freely available. Moreover, the M-AMBI performs more accurately than AMBI alone in low salinity habitats (Muxika et al., 2007; Borja et al., 2008).

**Biotic Index (Simboura and Zenetos, 2002).** The Biotic Index (BENTIX) is based on the AMBI index and uses the reduced soft-bottom macrozoobenthos classification consisting of three relatively wide EG’s.

Each group contains a list of indicator species receiving a score from 1 to 3 representing sensitive or indifferent to disturbance species (k-strategy); tolerant second-order opportunistic species (r-strategy) or those which may increase densities in case of disturbance; and first-order opportunistic species, respectively:

$$BENTIX = \left[ \frac{6 \times \%GI + 2 \times (\%GII + \%GIII)}{100} \right] \quad (2)$$

This index can range from 2 (for poor conditions) to 6 (at high status or reference sites). The main difference of this BENTIX formula, derived from the formula of AMBI index (Borja et al., 2000) is that the number of utilised ecological groups is reduced from five to three giving the same weight for the tolerant-second order opportunistic and the first-order opportunistic species (2). In this way the treated groups are posteriorly reduced to two: the sensitive and the tolerant. Also each ecological subgroup is weighted equally in relation to the others, resulting eventually to the ratio

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of 3 to 1 for the sensitive versus the tolerant group. Reducing the number of groups has the advantage of reducing uncertainty regarding the grouping (two groups instead of five) and also of increasing the simplicity of calculation.

The BENTIX was developed in the scope of the WFD (2000/60/EC) for the Mediterranean Sea. It has been successfully applied in assessment of organic pollution (Simboura and Zenetos, 2002; Simboura et al., 2005), oil spills (Zenetos et al., 2004) and dumping of particulate metalliferous waste (Simboura et al., 2007). Nevertheless, the BENTIX relies solely on the classification of organisms for organic pollution, and is being unable to accurately classify sites with toxic contaminations (Mari'n-Guirao et al., 2005). Limitations of the index use have been also met in case of transitional waters (estuaries and lagoons) where the natural conditions favour the presence of tolerant species in very high densities. In this case undisturbed lagoons or estuaries may appear with low quality status if the index is used (Simboura and Zenetos, 2002).

### 2.1.2 Multimetric indices for coastal waters of the Baltic Sea

**Benthic Quality Index (Rosenberg et al., 2004).** Benthic Quality Index (BQI) is based on macrozoobenthos species (or higher order taxa) abundance, species richness and species sensitivity values. It was designed to assess environmental status of Swedish marine waters following WFD (2000/60/EC) concept and its requirements (Rosenberg et al., 2004) (3). Currently Lithuania and Latvia have also adopted it for the assessments of the coastal waters status.

$$BQI = \left( \sum_{i=1}^n \left( \frac{A_i}{A_{tot}} * ES50_{0.05} \right) \right) \times \log_{10}(S + 1) \quad (3)$$

where  $A_i$  stands for the abundance of the species  $i$ ;  $A_{tot}$  is the sum of all individuals in a sample;  $ES50_{0.05}$  is the sensitivity value for the species  $i$ ; and  $S$  is the total number of species in a sample.

Macrozoobenthos species sensitivity values in BQI is based on Hurlbert diversity index ( $ES50$ ), which is calculated for each sample according to:

$$ES50 = \sum_{i=1}^s \frac{(N - Ni)! (N - 50)!}{(N - Ni - 50)! N!} \quad (4)$$

where  $S$  is the total number of species in a sample,  $N$  is the total number of individuals in a sample and  $N_i$  is the number of individuals belonging to the  $i^{th}$  species in a sample. Then, macrozoobenthos species sensitivity is calculated as  $ES50$  value, which corresponds the lower 5% limit of the cumulative species abundance distribution (i.e. 5<sup>th</sup> percentile of the species abundance) (Rosenberg et al., 2004) (Fig. 4). The assumption behind using  $ES50_{0.05}$  value to define species sensitivity is that sensitive species tend to occur more frequently at sites with high diversity (e.g. undisturbed sites) (Rygg, 2002) and get extinct under relatively low disturbance level. In contrast, tolerant species predominantly colonise sites with low diversity (e.g. disturbed sites) and hence are potentially more exposed to lower range of sensitivity values.

BQI applications were often restricted to a large and heterogeneous datasets characterized by a non-uniform sampling effort (Rosenberg et al., 2004). The requirement of species sensitivity values computation from large datasets causes severe limitations to the use of BQI index, particularly for areas of strong gradients, where it correlates with environmental variables, such as salinity (Zettler et al., 2007). Therefore, application of this index is sensitive to comprehensive datasets and quantification of local reference values, which reflects the environmental heterogeneity and justifies the range of index values (Rosenberg et al., 2004; Reiss and Kroncke, 2005; Labrune et al., 2006; Zettler et al., 2007).

**The Danish multimetric quality index (Borja et al., 2007).** The ecological quality of Danish coastal waters is assessed using a multimetric index DKI based on abundance, composition, and diversity of macrozoobenthos species. DKI uses the AMBI index where species sensitivity is classified according to Borja et al. (2000), log base 2 Shannon-Weaver diversity index and its maximum value in undisturbed conditions ( $H'$  and  $H_{max}$  respectively), number of species ( $S$ ) and the number of individuals ( $N$ ) (Borja et al., 2007):

$$DKI = \frac{\left(1 - \left(\frac{AMBI}{7}\right)\right) + \left(\frac{H}{H_{max}}\right)}{2} * \frac{\left(1 - \left(\frac{1}{N}\right)\right) + \left(1 - \left(\frac{1}{S}\right)\right)}{2} \quad (5)$$

The factors with  $N$  and  $S$  only have significant effect when number of individuals and species are  $< \sim 10$ . It has been tested in different pollution gradients both in Denmark (gradient of oxygen deficiency) and Sweden (gradient of pulp mill effluents) and found to reflect changes in the benthic communities due to pollution impact well (Josefson et al., 2009).

**The Brackish Water Benthic Index (Perus et al., 2007).** The Finnish Brackish Water Benthic Index (BBI) follows the theory that the biodiversity increases with the increasing distance from a pollution source along a gradient of disturbance (Pearson

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and Rosenberg 1978). It was used to classify the ecological status of the macrozoobenthic assemblages for the low-saline and species-poor Finnish coastal waters. The BBI includes relative abundance (%) of sensitive or tolerant species. The species sensitivity is the same as used for the Swedish BQI index (Rosenberg et al., 2004). The evaluation of sensitivity of each species is based on literature information (e.g. Borja et al., 2000) and expert judgment. The weights given for four sensitivity classes are: 1 – very tolerant to pollution; 5 – tolerant; 10 – pollution sensitive and 15 – very pollution sensitive.

The BBI is calculated as follows:

$$BBI = \frac{\left[ \left( \frac{BQI}{BQI \max} \right) + \left( \frac{H'}{H' \max} \right) \right]}{2} * \frac{\left[ \left( 1 - \frac{1}{AB_{tot}} \right) + \left( 1 - \frac{1}{S} \right) \right]}{2} \quad (6)$$

where, *BQI* is the Benthic Quality Index (Rosenberg et al., 2004), *H'* is the (log2-base) Shannon-Weaver diversity, *AB<sub>tot</sub>* is an abundance of species at station, and *S* the species richness.

The index considers the observed BQI and Shannon-Weaver diversity index (*H'*) values against their highest recorded values within individual water bodies (serving as reference values) and further deducts the obtained value considerably in case of low biodiversity or abundance. A value of 0 indicates conditions without macrozoobenthos organisms (azoic sediment) and value 1 indicates unpolluted bottom conditions.

Results from testing BBI and other indices on effects from fish farming showed that BBI correlates well with DKI and BQI but not with AMBI index (Perus et al., 2007). BBI, DKI and BQI indexes also showed a similar response to changes in oxygen saturation, organic matter, and species richness, while the AMBI index was insensitive to changes in these variables in low-saline species-poor regions.

**Marine Biotic Index Tool (Meyer et al., 2009).** The Marine Biotic Index Tool (MarBIT) is a multi-metric assessment system to rate the ecological status of macrozoobenthos communities in the German part of the Baltic Sea according to the requirements of the WFD (2000/60/EC). The assessment system is based solely on ecological principles and gives an assessment according to the four metrics or criteria defined by the WFD: macrozoobenthos composition, abundance, proportion of taxa sensitive to disturbance, and proportion of taxa that are pollution indicators. The approach uses a classification scheme with five classes, type or water body specific reference conditions, and is applicable to all habitats. The index is calculated from 10-20 pooled samples taken from any of three different substrates (soft bottom, phytal and hard bottoms) which are initially assessed separately (Meyer et al. 2009). The reference conditions are based on the available knowledge on the autecology of the species

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living in the Baltic coastal communities. This knowledge was taken from an extensive review of scientific literature and historical monitoring data. The fundamental principle of the assessment system is the fact that biological systems increase in complexity when they develop normally. On the other hand, disturbance from human activities reduces complexity. The four above mentioned WFD metrics for the assessment of the ecological status are used to measure this complexity and thus give an estimation of the complexity as a substitute for the ecological status. This is the ecological endpoint used in the MarBIT system.

MarBIT was tested against the Baltic Sea Pressure Index and inputs of nutrients with significant correlations (Meyer et al., 2009).

**Macrozoobenthos community index (Kotta et al., 2012).** The Estonian macrozoobenthos community index (ZKI) is based on the relative biomass, proportions of sensitive taxa and species richness on soft bottom substrate. The ZKI index divides the macrozoobenthos into three groups according to their sensitivity to an increasing stress (including eutrophication). The index also takes into account the species number at a station and compensates this diversity term for salinity of a site along the gradient. The compensation term is based on the waterbody-specific maximum value for species number. The macrozoobenthos community index is calculated by the following equation:

$$ZKI = [0.5 * (Class\ 1 + 2 * Class\ 2 + 3 * Class\ 3) - 0.5] * \left[ \frac{S}{S_{max}} \right] \quad (7)$$

where Class  $i$  is the ratio of the sum of the dry weights of the species belonging to the Class  $i$  to the total invertebrate biomass at the station;  $S$  is the number of species/taxa per grab;  $S_{max}$  is the waterbody-specific value of the maximum number of species per grab. Class 1 designates the opportunistic taxa that are able to form single-species associations or highly dominate communities under heavily disturbed conditions. Class 2 includes the taxa that are indifferent to or favoured by moderate eutrophication, but cannot tolerate heavily disturbed conditions. For the taxa of Class 3, eutrophication is unfavourable. The values of ZKI vary between 0 and 1, where 1 represents the healthy communities and 0 stands for the most deteriorated communities (Kotta et al., 2012).

The ZKI index was validated against nutrient loads (N and P concentrations) and by the Baltic Sea Pressure Index (BSPI). In spite of the index being adopted for soft bottoms in depths between 5 and 30 m sampled with Van-Veen or Ekman type benthic grabs, high variability in ZKI assessment results was found (Kotta et al., 2012).

All regional indices mentioned above were elaborated in order to meet WFD requirements, i.e. they account for the benthic abundance, composition as well as proportion of



tolerant and sensitive to disturbance taxa. However, there are two important constraints for applying these indices over broad spatial scales to the open sea areas of the Baltic Sea: (1) the dominance of a few individual species and their strong contribution to the natural variations in the macrozoobenthos abundance and (2) the problem with species sensitivity since a particular species may be classified as sensitive in one region and not in another (HELCOM MONAS report, 2009). Furthermore, as these Baltic regional indices are developed within the WFD, they mostly focus on the assessment of eutrophication effects. At this point an evaluation of the suitability, reliability and validation of the existing indices is a more urgent task than the development of new ones (Borja et al., 2008).

### **2.2 Requirements for the macrozoobenthos based environmental quality indexes**

Borja and Dauer (2008) have discussed the steps in a multimetric index development, which include: (i) selection of candidate metrics; (ii) metric combination; (iii) index validation; (iv) index application to different human pressures; (v) index interpretation; and (vi) index intercalibration.

The selection of effective indicators is a key stage in assessing the status and condition of a system. Several criteria have been suggested, including (but not limited to) scientific basis, responsiveness, range of applicability, concreteness, theoretical basis, public awareness, application costs, data requirements and availability, practicality, harmonization possibilities, accuracy, sensitivity, specificity and confidence (Rice and Rochet, 2005; Salas et al., 2006; Niemeijer and de Groot, 2008; Elliott, 2011). The evaluation methods and conceptual frameworks have been discussed to facilitate decision-making (Borja and Dauer, 2008; Kershner et al., 2011; ICES, 2013).

In the development of some benthic indices, calibration and validation data sets were initially available and samples were allocated between the two datasets randomly (e.g., Llanos et al., 2002) or based on sampling year (e.g. Paul et al., 2001). More commonly after the index development, newly collected data are used as validation data. In such cases strong putative gradients are deliberately selected (e.g. Borja et al., 2000, 2003, 2006; Muxika et al., 2005; Quintino et al., 2006).

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 to the water quality parameters (dissolved oxygen, particulate and total organic carbon) was assessed using linear regression (Simboura and Zenetos, 2002). 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. Statistically significant cor-



relations were found between BQI and annual mean of oxygen, chlorophyll a and total nitrogen concentrations (Osowiecki et al., 2008). Recently Borja et al. (2015) have ranked 35 benthic quality indices used in different countries to evaluate the impact produced by 15 different human pressures (including multipressure, aquaculture, sewage discharges, eutrophication, physical alteration, chemical pollution, climate change). The ranking was carried out by taking into account the coverage area of biogeographical provinces, number of citations for testing an index against selected pressures and number of citations for significant index correlation with a pressure. The highest performance was found for AMBI, M-AMBI, BENTIX, BQI and BOPA.

### 2.3 Macrozoobenthos species sensitivity assessment methods

The concept of benthic sensitivity was inspired by the paradigm of macrozoobenthos succession in a context of organic enrichment in the marine environment (Pearson and Rosenberg, 1978). This paper stimulated extensive discussions about the concepts of indicators and indices used in the analysis of soft-bottom macrobenthic communities (e.g. Laine et al., 1997; Kotta et al., 2009). Pearson and Rosenberg (1978) argued that unidirectional stress caused by an increasing intensity of a particular disturbance will result in adaptation by an individual within its abilities to respond first, and then it will be replaced by another better adapted individual able to respond to that particular stress. Beyond this level the species will be replaced by other species better adapted to the new conditions. However, the increase of organic matter is only one of several possible pollution outcomes in the sediment; and other substances (e.g. metals, hydrocarbons) or physical disturbances (deposition of dredged material, bottom trawling, siltation) can also interfere and instantaneously shape the structure of benthic communities along the eutrophication gradients. In this case the dynamics of various species or ecological groups becomes extremely complex depending on a set of acting stressors, structure of impacted communities and background spatio-temporal variability of a benthic environment.

Species sensitivity is determined as a product of the likelihood of a damage (also termed intolerance or resistance) due to a pressure and the rate of (or time taken for) recovery (also termed recoverability, or resilience) once the pressure has abated or been removed (Laffoley et al., 2000). A species is defined as very sensitive when it is easily adversely affected by human disturbance (e.g. low resistance) and recovery is only achieved after a prolonged period, if at all (e.g. low resilience or low recoverability) (Laffoley et al., 2000; OSPAR, 2008). Human activities in the marine environment result in a number of pressures, which may result in an impact on environmental components that are sensitive to the pressure. Pressures have been defined as ‘the mechanism through which an activity has an effect on any part of the ecosystem’ (Robinson et al., 2008). Pressures can be physical, chemical or biological. Different pressures can result

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in the same impact, for example, habitat loss and habitat structure changes can both result in the mortality of benthic invertebrates (Robinson et al., 2008). Species will differ in their ability to resist different pressures based on the type of pressure, the extent, duration and magnitude of the pressure and the degree of exposure. The timing of the pressure exposure can also be significant, in relation to species' life cycles, reproduction, recruitment or even season or time of day with some species being active and/or present in different areas at different times. Different life stages of an organism may also vary in sensitivity to pressures. Sensitive species frequently survive within a narrow range of environmental conditions and disappear from polluted areas and zones undergoing environmental change. Such species are typically limited in abundance, restricted in distribution, or are particularly sensitive during development and rely on specific habitat conditions (Dauvin, 2010). Species sensitivity to anthropogenic disturbance may also vary along the environmental gradients such as depth, salinity, wave exposure and substrate type (Villnäs and Norkko, 2011), some species are even capable to adapt or change their performance under different stress conditions (Remane, 1958).

Different methods and approaches have been used to assess macrozoobenthos species sensitivity and majority result in the classification of ecological groups containing species of different sensitivity to a given disturbance, e.g. organic enrichment (Pearson and Rosenberg, 1978; Rumohr et al., 1996), industrial waste (Leppäkoski, 1980), thermal pollution (Zettler et al., 2013), dredge spoil dumping (Olenin, 1992), bottom trawling (Kaiser et al., 2000). Still, empirical evidence of macrozoobenthos sensitivity relation to human pressures is largely lacking, while importance of existing natural gradients for species of different sensitivity groups is restricted to few regional studies (e.g. Zettler et al., 2007; Schiele et al., 2016).

Species sensitivity values are commonly used in various indices for assessing status of marine environment as in the WFD (2000/60/EC) and more recently also within the MSFD (2008/56/EC). One of the most challenging aspects of benthic indices has been the identification of reliable measures of the species' sensitivity to various magnitudes and different kinds of disturbances. Marine benthic fauna encompasses thousands of species and most of them occur at low densities. Scientific information about the ecology of many species is limited, which makes it hard to assign sensitivity values based on documented knowledge. Species sensitivity characteristics can be evaluated from existing knowledge reported in the literature, using expert judgment or deriving information through data analysis of macrozoobenthos structure under various effects (Mearns and Word, 1982; Borja et al., 2000; Muxika et al., 2007; Bellan, 2008; Pearson and Rosenberg, 1978; Rosenberg et al., 2004; Leonardsson et al., 2009).

Soft-bottom macrozoobenthos species respond to environmental or human stress by means of different adaptive strategies. Gray (1979) summarized these strategies by classifying species into three ecological groups: r-strategy species with a short life-span, fast growth, early sexual maturation and larvae throughout the year, i.e.

typical opportunist species; k strategy species with a relatively long life, slow growth and high biomass; and T strategy stress tolerant species, not affected by alterations. Mearns and Word (1982) suggested species classification based on trophic groups: suspension feeders; carrion feeders (e.g. carnivorous, omnivorous and necrophagous species); surface deposit feeders, species that are both suspension feeders and surface deposit feeders, subsurface deposit feeders that feed on sedimentary detritus and bacteria. Benthic species can also be classified according to their biological traits, such as longevity, reproduction type, life history, morphology and behaviour characteristics (Bremner et al., 2003). The presence or absence of a species in a sample, such as a first-order opportunistic species or a sentinel species, can be enough to indicate degradation or the effect of pollution on a benthic community (Bellan, 2008).

Borja et al. (2000, 2003, 2008) summarised work of Pearson and Rosenberg (1978) and Grall and Glemarec (1997) and classified more than 2000 benthic taxa into five ecological groups based on the dynamics of species relative abundance. Three ecological groups of the macrozoobenthos are used in the BENTIX index according to the degree of sensitivity towards pollution (Simboura and Zenetos, 2002). In this case the information classifying the species into the ecological groups was derived from 35 literature sources providing the ecological characterisation of species for different regions (e.g. Borja et al., 2000; Corbera and Cardel, 1995; Simboura and Nicolaidou, 2001). In contrast, Leonardson and his colleagues (2015) used a semi-quantitative scale for the characterisation of species sensitivity: instead of *ES50* values for species sensitivity values they used the observed number of species in each sample. The regional and fully quantitative attempt to describe variability of 329 species sensitivity was carried out using 19 combinations of geographic characteristics and the same calculation method for the entire Baltic Sea (Schiele et al., 2016). The result emphasises the necessity of assigning different sensitivity values for majority of species for various stretches of the environmental gradients and physiogeographic regions (subregions, classes of salinity, depth, sampling gear). Other authors, however, used expert based sensitivity classifications assigning weights from 1 to 15 for species belonging to one of four sensitivity classes (Osowiecki et al., 2008; Leonardson et al., 2009).

Zettler et al. (2013) also pointed out that static indicator species lists should be applied with caution for environmental quality assessments. The importance of interacting environmental variables, the possible existence of cryptic species and different sensitivity during various life stages of the species should not be neglected when using static indicator lists. Consequently, the following aspects should be carefully considered when applying the static indicator based quality indices: (1) species sensitivity may change along environmental gradients and between different biogeographic regions; (2) there is a risk of including sibling or cryptic species in calculating the sensitivity value of a species.



# 3

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## Material and Methods

### 3.1 Study area

This study was performed in the Lithuanian marine area (LMA, includes Territorial waters and EEZ) located within the south-eastern part of the Baltic Sea. Lithuanian EEZ occupies 6426.6 km<sup>2</sup>, while territorial sea covers more than 3 times less and is restricted to the area of 1849 km<sup>2</sup>. In the south the Lithuanian EEZ borders with the Russian Federation, while in the north and west with Latvian and Swedish waters, respectively.

A large part of LMA belongs to Klaipėda-Ventspils plateau and slopes of the northern Gdansk Basin. Three comparatively deep areas extending below the halocline in the Lithuanian EEZ are depressions of Gdansk and Gotland basins and palaeo valley of the Nemunas River. The central part of the LMA is relatively flat, determined by south-westerly inclined slopes of Gdansk Basin. The average depth of LMA is approximately 50 m with the maximum of 125 m reached along the Gotland Basin slope.

Coastal area is delineated by depth of 20-30 m and differ considerably from the open Baltic Sea according to the ecological conditions, such as depth and salinity range, water temperature, predominant bottom substrate, wave exposure and anthropogenic pressures (Olenin and Daunys, 2004).

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#### 3.1.1 Characteristics of the coastal waters

Coastal waters along the Lithuanian coastline in the south-eastern part of the Baltic Sea are largely affected by the plume of the Curonian Lagoon, which enters the sea through the narrow (150 m wide) Klaipėda Strait. The plume area in the Lithuanian Baltic Sea coastal waters covers the whole territorial sea, however mainly is directed towards the mainland coast to the north (Vaičiūtė et al., 2012).

Although the surface salinity in the Lithuanian marine waters is generally in range between 7 and 8, depending in hydrometeorological situation it may decrease to almost freshwater near the mouth of the Curonian Lagoon salinity and salinity gradient may extend for tens of kilometers out into the Sea (Vaičiūtė et al., 2012). In the surface sediment coarse and medium sands typically occur from the shore down to 20 m depth and such areas occupy approximately 80% of the total coastal area (Bitinas et al., 2004). The rest are mixed bottoms mainly consisting of boulder, pebble and sand patches restricted to the north off Klaipėda Strait (Olenin and Daunys, 2004).

Vertical distribution of temperature is season dependent. The water is cold and homogeneous from December through March due to the intensive convection. In the summer, the thermocline is formed at 20-30 m depths, which separates warm upper water layer and relatively cold deep water. The temperature gradient between near-bottom waters in coastal and offshore areas can reach 12-15°C.

#### 3.1.2 Characteristics of the open marine waters

In the open waters the near-bottom salinity above the halocline ranges from 6 to 8. The centre of the halocline is found at 74 m with mean boundaries of 64-90 m, where salinity increases from around 8 to 10. The salinity in the active sub-halocline water layer (90-130 m) can reach over 12 (Omstedt et al., 2014).

Patches of pebble/gravel deposits occur on sites down to 60 m, but in general, this type of bottom is common only for the coastal slopes in depths less than 30 m (Olenin and Daunys, 2004). Types of soft sediment change from fine sands occurring down to 50 m to coarse aleurites extending down to 70-90 m depth as well as fine aleurite and aleurite-pelitic mud at the slopes of the Eastern Gotland Basin between 80 and 100 m. Below 90-100 m the main type of bottom sediment is pelitic mud, which covers the slopes and floor of the Gotland Deep.

The deep water in the central Baltic Sea basin tends to stagnate for periods of several years. Remineralization of deposited organic material may facilitate anoxic conditions and lead to the formation of considerable concentrations of hydrogen sulphide. Strong seawater inflows from the North Sea may cause prolonged vertical salinity stratification and the lack of efficient oxygenation in the sub-halocline area. Hypoxic conditions with salinity around 1 ml l<sup>-1</sup> are present in the active sub-halocline layer.

Also, the oxygen concentration drops significantly from 6-9.5 ml l<sup>-1</sup> (saturation 70-100%) to 2 ml l<sup>-1</sup> (< 20% saturation) going down from the upper water layer to the halocline range (Olenin, 1997).

Macrozoobenthos of the southern slope of the Eastern Gotland Basin is subject to permanent fluctuations of oxygen concentration and salinity. The lack of oxygen leads to the impoverishment and subsequent disappearance of the macrozoobenthos community, while low salinity precludes the dispersal of some halophile species into the upper part of the seabed slope (Olenin, 1997).

#### 3.1.3 Anthropogenic disturbances at the study area

This study is addressing several important human related disturbances in the Lithuanian part of the south-eastern Baltic Sea: eutrophication, dredge spoil dumping and bottom trawling (Fig. 3).

**Eutrophication.** The Baltic Sea represents the world's largest brackish-water sea area influenced by a limited inflow of marine, fully saline water from the North Sea and a high input of fresh water from the rivers. Phytoplankton quantity is a direct proxy of eutrophication due to a strong link to the increase of nutrient concentrations. In some areas nutrient concentration increase is supplemented by an internal nutrient loading from the bottom, accelerated by oxygen depletion. In turn, the phytoplankton increase adds to the oxygen depletion, when sedimenting to the bottom and causing a vicious circle of eutrophication. The increase of Chl-*a*, a proxy of phytoplankton biomass, in the water column is dependent on nutrient concentrations, and thus linked strongly to anthropogenic nutrient loads from land and air (HELCOM, 2009).

In the context of the Lithuanian marine waters, the Nemunas River runoff is a major contributor to the total riverine runoff and most of the nutrient loads from the land (Ferrarin et al., 2008). Total fresh water runoff from the Curonian Lagoon to the Baltic Sea through the Klaipėda Strait is approximately 27.7 km<sup>3</sup>/year (Jakimavičius and Kovalenkoviėnė, 2010). This runoff frequently forms a spatio-temporally unstable riverine plume in the territorial sea, which is traced through elevated Chl-*a* and changed optical properties of water masses. Chl-*a* in the plume area varies from 4.7 to 156.2 mg/m<sup>-3</sup> (mean 38.4±31.5 mg/m<sup>-3</sup>), while outside the plume Chl-*a* is lower and ranges from 2.2 to 20.2 mg/m<sup>-3</sup> (mean 5.7±4.5 mg/m<sup>-3</sup>) (2005-2011 data) (Vaičiūtė et al., 2012). Mean salinity of the plume area is also significantly lower than the salinity of the adjacent coastal waters and highly correlates with the absorption of coloured dissolved organic matter (CDOM) (Vaičiūtė et al., 2012).

Cyanobacterial blooms have been reported from the open Baltic Sea already in the 19th century, but their intensity and frequency seem to increase (Finni et al., 2001). During the two recent decades, an intensive bloom caused by *Aphanizomenon flos-aquae* was observed, while during summer season of period 1986-1989 and later

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1994-1996 seasons, it reached hyperbloom conditions (Olenina and Olenin, 2004). Bloom-forming cyanobacteria play an important role in the Baltic Sea ecosystem because of their nitrogen fixation capabilities and their toxicity. Surface blooms of *A. flos-aquae* and *Nodularia spumigena* occur regularly in summer in the Baltic Proper. The most abundant species *A. flos-aquae* is constantly presented in the phytoplankton throughout the year, but rapidly increases in abundance (by 100-1000 fold) when the water temperature reaches 20°C. The bloom usually lasts until the end of October/beginning of November. According to Vaičiūtė and her colleagues study (2012) in the estuarine plume area of the Lithuanian coastal zone the biomass of *A. flos-aquae* was 24 mg/m<sup>3</sup> and according to Reimers (1990) scale reached the level of intensive bloom. In the Lithuanian waters during summer period (June–September) the majority of phytoplankton taxa belong to Cyanophyceae and Chlorophyceae, also Bacillariophyceae and Dinophyceae (Vaičiūtė et al., 2012).

**Dredge spoil dumping.** There are two dredge spoil dumping sites in the Lithuanian marine waters, where 2 km<sup>2</sup> shallow dredge spoil dumping area is open since 1996 and located at depths of 28-35 m approximately 10 km from the coastline north-west of the mouth of the Curonian lagoon. Only fine sand and aleurites are dumped in this area. Larger dredged spoil dumping area of 17.8 km<sup>2</sup> is operated since 1986 and located at depths of 45-50 m approximately 20 km from the coastline, south-west of the mouth of the Curonian lagoon. Approximately 10 mln m<sup>3</sup> of dredged material, mainly moraine mixed with clay (70%) was deposited in the area from 2000 to 2010. Due to its relatively remote location, this dredge spoil dumping area is rarely affected by reduced salinity of the Curonian lagoon plume area (Vaičiūtė et al., 2012). Enhanced concentrations of Cd, Cu, Pb have been recorded in this dredge spoil dumping site during the environmental monitoring (e.g. Marine Research Department, 2015). Permanent effects on macrozoobenthos and seabed topography have been also reported in the designated area of the dredge spoil dumping site (Olenin, 1992).

**Bottom trawling.** The extent of the bottom trawling in the Lithuanian exclusive economic zone was currently investigated by analysing the VMS data for the recent decade (Daunys et al., 2016). Bottom trawling occurs in approximately 2/3 of the total EEZ area with the annual average intensity (i.e. relative coverage of the area by bottom trawling activity) of 13.7±0.7% for 1x1 nm grid. This study was performed in the most intensively trawled areas located in the southwestern part of the EEZ at depths of 60-65 m, where the annual trawling intensity was typically higher than 50% and exceeded 200% in some years.



## 3.2 Description of macrozoobenthos samples and data

### 3.2.1 General information on sampling, analytical procedures and compiled datasets

All macrozoobenthos samples used in the analysis of this study were collected from the soft-bottom habitats at depths ranging from 10 to 70 m. Abundance (ind m<sup>-2</sup>) data were derived from 587 samples collected at 169 sites in the LMA during the period from 2002 to 2015. These data were gathered in the framework of five short-term projects and three long-term monitoring programmes carried out with different sampling intensity and during different years (Table 1). Eight datasets were collated from the above mentioned information sources in order to implement study tasks (Table 2).

Irrespective of the research programmes and survey, all samples were taken using Van-Veen grab (0.1 m<sup>2</sup> sampling area), sieved on-site through a 0.5 mm mesh and preserved with 4% formaldehyde solution. Further processing of samples took place in the laboratory according to HELCOM recommendations (HELCOM, 2015) where organisms were sorted using a stereoscopic microscope and the specimens were determined and quantified to the lowest possible taxonomic level (e.g. species or genus).

**Table 1. Information sources used to generate datasets for macrozoobenthos analysis in this study.**

*1 lentelė. Makrozoobentosų gausumo duomenų, naudotų analizėms, šaltiniai.*

| Data source number | Project or sampling program                                                                                                            | Responsible institution                                   | Time period |
|--------------------|----------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|-------------|
| 1.                 | National monitoring                                                                                                                    | Environment Protection Agency, Marine Research Department | 2002-2012   |
| 2.                 | Environmental monitoring of Butinge oil terminal                                                                                       | Klaipėda Seaport Authority                                | 2002-2012   |
| 3.                 | Environmental monitoring of Klaipėda Seaport                                                                                           | Klaipėda Seaport Authority                                | 2002-2012   |
| 4.                 | AVEC project (The use of wind energy in the open waters off Šventoji-Palanga)                                                          | Klaipėda University                                       | 2012        |
| 5.                 | DENOFLIT project (Inventory of marine species and habitats for development of Natura 2000 network in the offshore waters of Lithuania) | Klaipėda University                                       | 2013        |

### 3. Material and methods

| Data source number | Project or samling program                                                                                                                                       | Responsible institution | Time period |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------|
| 6.                 | NFM project (A system for the sustainable management of Lithuanian marine resources using novel surveillance, modeling tools and an ecosystem approach)          | Klaipėda University     | 2009        |
| 7.                 | TRIPOLIS project (Bottom trawling intensity and impacts on the benthic ecosystem of the Lithuanian Baltic Sea)                                                   | Klaipėda University     | 2015        |
| 8.                 | Environmental impact assessment of sand extraction activities in the central Baltic Sea off Juodkrante – Preila and beach nourishment in the vicinity of Palanga | Klaipėda University     | 2008        |

Generally, coordinates of sampling sites were predetermined before surveys and reflected aims of concrete research programmes. In contrast, sampling sites for bottom trawling dataset were derived after locating trawl track positions on the seabed by a multibeam echosounder and side scan sonar and choosing appropriate sampling site with respect to this information. In both cases the sampling sites within the tracks (impacted sites) and outside the tracks (control sites) were possible to locate precisely by combining a high resolution acoustic map, dGPS positioning and vessel dynamic positioning system.

Surface water Chl-*a* (mg m<sup>-3</sup>) data for the coastal waters was retrieved from the cloud free MEdium Resolution Imaging Spectrometer (MERIS) (FR, 300 m), the ENVISAT satellite of the European Space Agency. After 2nd reprocessing MERIS Level 1b images firstly were corrected to account for the difference between actual and nominal wavelengths of the solar irradiance in each channel (Fomferra and Brockmann, 2006) with the Smile tool (1.2.101 version) of the BEAM VISAT (4.8.1) software provided by Brockmann Consult/ESA, in order to perform an irradiance correction for all bands. Later, the images were processed using four different plug-in optical processors of the BEAM VISAT (4.8.1) software in order to retrieve Chl-*a* for approximately 3x3 km<sup>2</sup> regions around macrozoobenthos sampling sites during 2005-2011. The average Chl-*a* for June-August period was calculated and used in the further analysis.

TP and TN (µg/l) data (0-10 m surface layer, June-August period of 2005-2011) for the coastal waters and plume area of macrozoobenthos sampling sites were derived from the national monitoring dataset (Marine Research Department, Environmental Protection Agency) and used in the further analysis.

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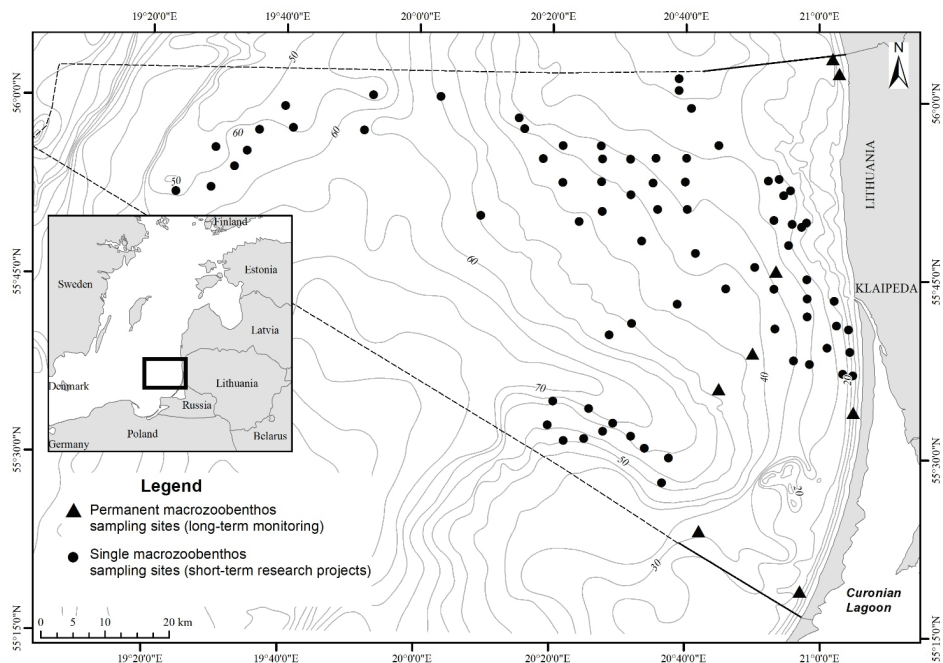


Figure 1. Distribution of macrozoobenthos sampling sites (long-term monitoring and short-term research projects) used for compilation of dataset to analyse dependence of species sensitivity values on depth.

*1 paveikslas.* Makrozoobentos mėginių, naudotų analizuoti gylio poveikį rūšių jautrumo vertėms, rinkimo vietų pasiskirstymas (rinktų ilgalaikio monitoringo ir trumpai vykdytų projektų metu).

The dataset of macrozoobenthos distribution along the depth gradient was compiled for the analysis of depth importance for species sensitivity values and BQI changes (Table 2, Fig. 1). Monitoring data for 8 permanent stations represented information on interannual variability for the same depth.

Two datasets of coastal waters macrozoobenthos communities were compiled for testing community structure effects on species sensitivity values and BQI (Table 2, Fig. 2). Only samples dominated by bivalve *Limecola balthica* or polychaete *Marenzelleria* spp. were included into a corresponding dataset. The samples were assigned to the macrozoobenthos community according to the biomass dominant species, which accounts for more than 40% of the total macrozoobenthos biomass in a site represented by a single sample.

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**Table 2.** Description of macrozoobenthos datasets used for analysis in this study.

**2 lentelė. Makrozoobentos duomenų rinkinių, naudotų analizėms, apibūdinimas.**

| Description of macrozoobenthos dataset                                                                                   | Study year | Depth range (m) | Number of samples | Number of sampling sites | Data source* | Purpose of dataset use                                                                                                                                                                            |
|--------------------------------------------------------------------------------------------------------------------------|------------|-----------------|-------------------|--------------------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Macrozoobenthos taxonomic composition, abundance of taxa and depth data                                                  | 2002-2013  | 10-70           | 139               | 92                       | 1;2;3;4;5;6  | Analysis of species sensitivity values dependence on depth and its role for BQI values                                                                                                            |
| Macrozoobenthos taxonomic composition, abundance of taxa data from samples dominated by bivalve <i>L. balthica</i>       | 2002-2012  | 10-30           | 54                | 21                       | 1;2;4;6;8    | Testing consistency of species sensitivity values and subsequently calculated BQI for different macrozoobenthos communities (bivalve <i>L. balthica</i> and polychaete <i>Marenzelleria</i> spp.) |
| Macrozoobenthos taxonomic composition, abundance of taxa data from samples by polychaete <i>Marenzelleria</i> spp.       | 2002-2012  | 10-30           | 51                | 24                       | 2;3;6;8      |                                                                                                                                                                                                   |
| Macrozoobenthos taxonomic composition, abundance of taxa and Chl- <i>a</i> concentration data at the eutrophication area | 2002-2012  | 10-20           | 65                | 4                        | 1;2          | Analysis of species sensitivity values dependence on disturbance level and disturbance type; its importance for BQI                                                                               |
| Macrozoobenthos taxonomic composition, abundance of taxa data at the dredge spoil dumping area                           | 2002-2012  | 40-50           | 118               | 13                       | 3            |                                                                                                                                                                                                   |

### 3. Material and methods

| Description of macrozoobenthos dataset                                                    | Study year | Depth range (m) | Number of samples | Number of sampling sites | Data source* | Purpose of dataset use                                                          |                                                                        |
|-------------------------------------------------------------------------------------------|------------|-----------------|-------------------|--------------------------|--------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Macrozoobenthos taxonomic composition, abundance of taxa data at the bottom trawling area | 2015       | 60-65           | 48                | 48                       | 7            |                                                                                 |                                                                        |
| Macrozoobenthos taxonomic composition, abundance of taxa data at the coastal waters       | 2002-2012  | 10-20           | 58                | 4                        | 1;2          | Testing BQI response to the eutrophication parameters Chl- <i>a</i> , TP and TN | BQI validation and determination of the water quality class boundaries |
| Macrozoobenthos taxonomic composition, abundance of taxa data at the plume area           | 2002-2012  | 10-20           | 54                | 2                        | 1            | Testing BQI response to the eutrophication parameters Chl- <i>a</i> , TP and TN |                                                                        |

\*-source numbering refers to numbers provided in Table 1.

Three macrozoobenthos disturbance datasets were compiled based on samples distribution along the gradients of three dominant pressures: eutrophication, dredge spoil dumping and bottom trawling (Table 2, Fig. 3). The dataset of macrozoobenthos abundance response to the eutrophication (i.e. elevated summer Chl-*a*) in the coastal waters includes samples taken at 4 permanent environment monitoring sites. This dataset was supplemented with Chl-*a* data for corresponding years (see page 34 for details on data characteristics). The dataset of macrozoobenthos response to the dredge spoil dumping disturbance was formed on the data from the samples taken at 13 permanent monitoring sites sampled for the 10 year period, while the dataset of macrozoobenthos

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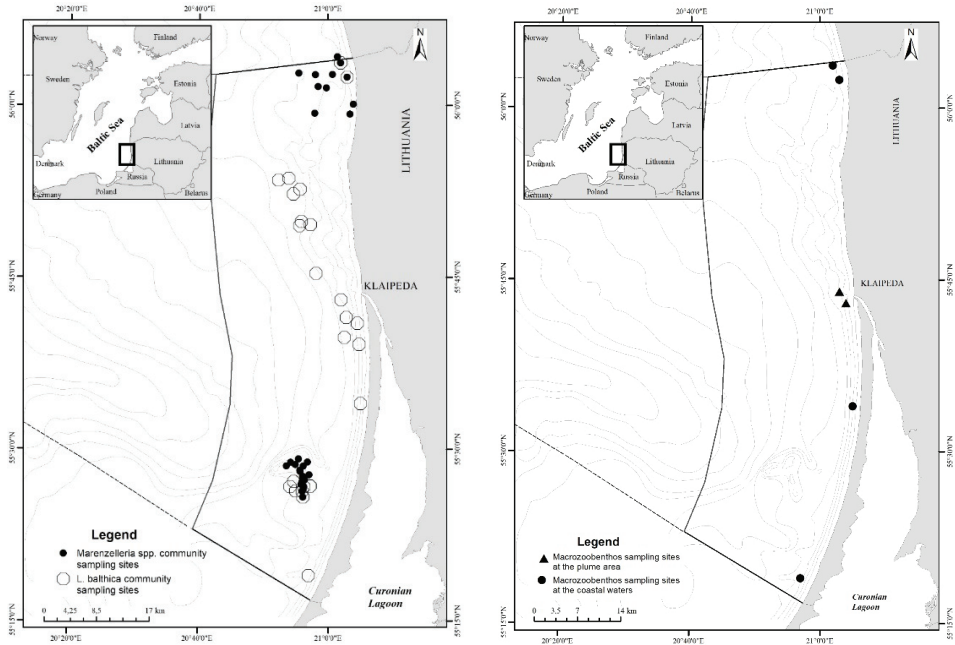


Figure 2. Distribution of macrozoobenthos sampling sites used for compilation of a dataset to: i) test consistency of species sensitivity values between two macrozoobenthos communities dominated by *L. balthica* and *Marenzelleria* spp. (left); and ii) perform of the BQI validation (right).

2 paveikslas. Makrozoobentos mėginių, naudotų rūšių jautrumo verčių kaitos tarp dviejų skirtingų bendrijų analizei, (kairėje) ir Bentoso kokybės indekso validacijai (dešinėje), rinkimo vietų pasiskirstymas.

response to the bottom trawling activity consists of the samples collected one time during the short-term research project in June, 2015 (Table 2, Fig. 3).

The dataset of macrozoobenthos abundance and calculated BQI response to the eutrophication disturbance parameters expressed by Chl-*a*, TP and TN (see page 34 for details on data characteristics) was formed for the coastal waters and plume area (Table 2, Fig. 2). These parameters were chosen as the “direct measures” of eutrophication, suggested among others within the MSFD (Ferreira et al., 2011).

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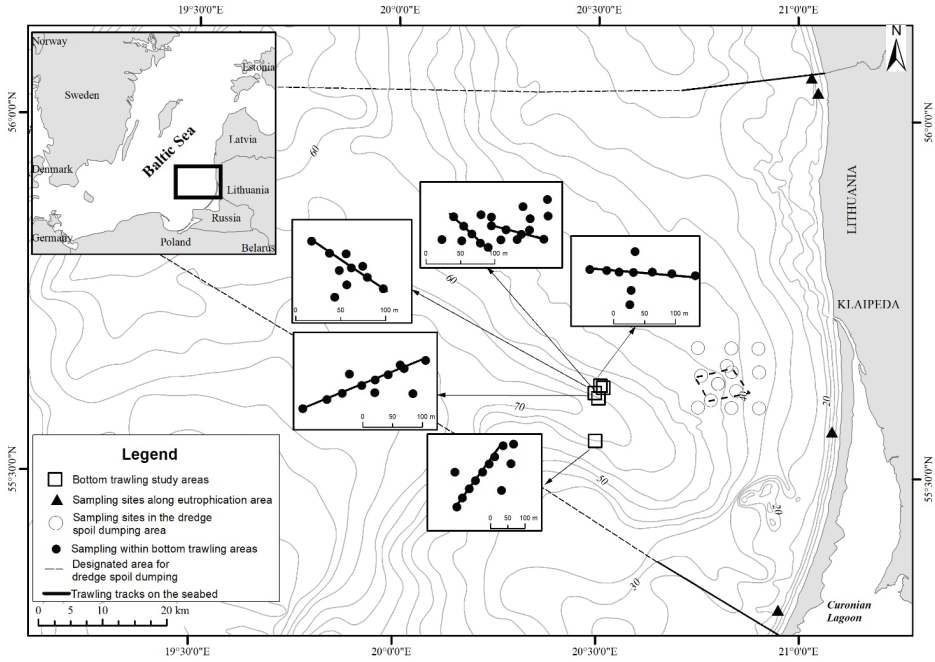


Figure 3. Macrozoobenthos sampling sites used for compilation of dataset to analyse species sensitivity values dependence on eutrophication (Chl-*a* elevated), dredge spoil dumping and bottom trawling disturbance.

3 paveikslas. Makrozoobentoso mėginių, naudotų eutrofikacijos, grunto pylimo ir dugninio tralavimo poveikių rūšių jautrumo verčių analizei, rinkimo vietų pasiskirstymas.

#### 3.2.2 Calculation of Benthic Quality Index values

The original version of BQI is known to be sampling effort dependent (Rosenberg et al., 2004) (3), therefore its adjusted form according to Fleischer and Zettler (2009) was applied for the data analysis in this study:

$$BQI = \left( \sum_{i=1}^n \left( \frac{A_i}{A_{tot}} \times ES50_{0.05} \right) \right) \times \log(ES50 + 1) \times \left( 1 - \frac{5}{5 + A_{tot}} \right) \quad (8)$$

In the equation above,  $n$  denotes the observed number of species  $A_i$  stands for the abundance of the species  $i$ , and  $A_{tot}$  is the sum of all individuals in the sample. Finally,  $ES50_{0.05}$  is the sensitivity value for the species  $i$  and  $ES50$  is Hurlbert diversity index (i.e. the estimated species number for 50 random individuals in the sample) calculated

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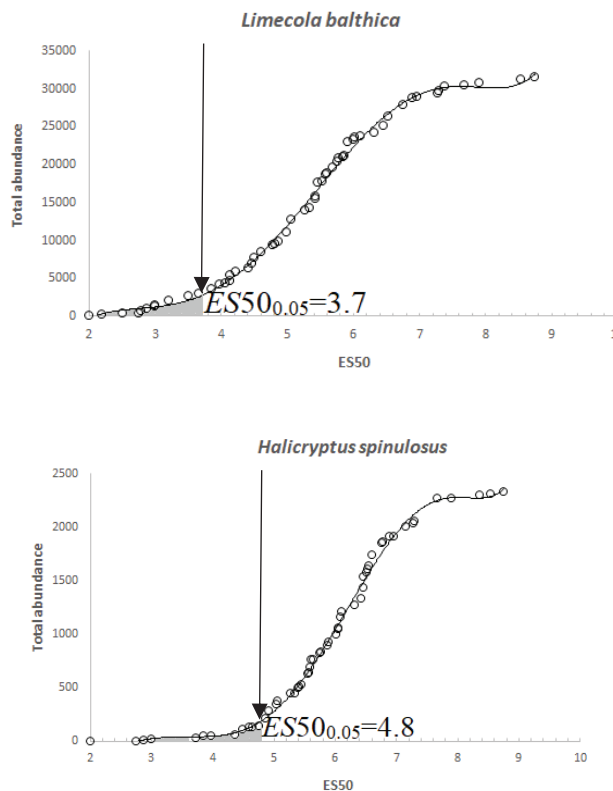


Figure 4. Examples of abundance distributions of bivalve *L. balthica* and priapulid *Halicryptus spinulosus* plotted against *ES50* values for samples from the dredge spoil dumping area dataset. Shaded areas indicate 5% of the species abundance and their right side boundaries in relation to the *ES50* values corresponding to the sensitivity value of the given species ( $ES50_{0.05}$ ). Following this approach, the sensitivity values for *L. balthica* and *H. spinulosus* is estimated at 3.7 and 4.8 respectively.

4 pavyzdys. Dvilgeldžio *L. balthica* ir priapulido *Halicryptus spinulosus* gausumo pasiskirstymas pagal mėginių *ES50* reikšmės grunto pylimo rajone. Pilkos zonos parodo 5% rūšies gausumo vertės santykį su mėginių *ES50* verte, kuris išreiškiamas kaip jautrumo vertė ( $ES50_{0.05}$ ). Šiame pavyzdyje *L. balthica* ir *H. spinulosus* rūšims nustatytos atitinkamai 3,7 ir 4,8 jautrumo vertės.

according to formula (4) (see page 21 in chapter 3.1.2). *ES50* and BQI values were calculated for every sample, and  $ES50_{0.05}$  values for every species using R v3 statistical computing environment (R-project., 2014, R-script developed by A. Darr).

Species sensitivity values were calculated for all higher order taxa identified to the species level, as well as for *Marenzelleria* spp., *Ostracoda* undet., *Oligochaeta*



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undet., *Hydrobia* sp. and *Nemertini* sp. Although a minimum of 20 samples was set for species occurrence in a dataset to estimate its true sensitivity value (Rosenberg et al., 2004), sensitivity values in this study were estimated for all species occurring in at least 8 samples. Moreover, in contrast to the earlier practice to analyse only samples with higher than 50 ind m<sup>-2</sup> density (Rosenberg et al., 2004), this study involved all samples with non-zero species density. These lower thresholds in species occurrence and abundance were used in order to keep the number of samples in the analysis as high as possible and cover wider range of a species response along the analysed gradient. The validity of samples with less than 50 individuals (n=12) was justified by the identical *ES50* values and number of species for the corresponding samples, therefore the original *ES50* values were used for further sensitivity calculations in order to extend the representation of disturbance gradient by the dataset.

#### 3.2.3 Analysis of compiled datasets and statistical procedures

The datasets were analysed using various parametric (Student's t-test) and non-parametric (Kruskal Wallis, Mann-Whitney (employed in the package "Commander" for the statistical software R version 2.15.1) (Wilcoxon signed-rank) tests following the results of normality (Shapiro-Wilk test) and heteroscedastity tests (Levene test) (Statistical package for the Social Science (SPSS) software, 19.0 version, 2010). Various transformations of macrozoobenthos abundance data were applied for similarity based ordination methods in order to get the highest correspondence between similarity values and distances in the plots (i.e. the lowest stress value) as well as the best discrimination (i.e. maximum dissimilarity) of samples.

**Depth gradient dataset analysis.** Macrozoobenthos abundance, species distribution depth limits (upper and lower number of boundaries), occurrence along the depth gradient and total number of species were used for the analysis of the benthic communities structure along the depth range from 10 to 70 m. The upper boundary number-number of species limits is where species are identified and occur for the first time along the analysed depth interval. The lower boundary number-number of species limits is where species are no longer identified in the greater depths. The non-metric multidimensional scaling (nMDS) analysis using Primer v6 software (Clarke and Warwick, 2001) based on macrozoobenthos abundance was used to justify the classification of samples across different depth intervals (10-30 m, 30-50 m, 50-70 m). The statistics of similarity percentages (SIMPER) and analysis of similarities (ANOSIM) were used to justify and describe the classified depth intervals.

**Community structure dataset analysis.** The nMDS was employed to discriminate macrozoobenthos abundance samples between two dominant communities (*L. balthica* and *Marenzelleria* spp.).

**Disturbance dataset analysis.** In the analysis of eutrophication (Chl-*a*), dredge spoil dumping and bottom trawling effects on species sensitivity the values were counted for two different groups of samples in the corresponding dataset: the control (includes samples from undisturbed sites only) and full (includes samples from undisturbed and impacted sites) datasets. For all analysed anthropogenic disturbance areas, the nMDS was employed using Primer v6 software to distinguish between the control and impacted samples. This classification was based on a similarity matrix derived using Bray-Curtis similarity index.

**Eutrophication (Chl-*a* elevated) area.** The spatial distribution of Chl-*a* in the coastal waters was used to characterise an eutrophication gradient under the assumption that an increase of phytoplankton correlates with an impact on the macrozoobenthos abundance. The analysis of remote sensing Chl-*a* data showed considerably higher range of Chl-*a* values (6-8 mg m<sup>-3</sup>) in the northern part of the studied coastal waters (largely overlapping with the Curonian lagoon plume area), compared to Chl-*a* in the southern part (2-4 mg m<sup>-3</sup>). Therefore, the northern part of the coastal waters area was arbitrarily characterised as the impacted area in the context of the southern part, which was characterised as a control area. The nMDS analysis of the macrozoobenthos abundance samples were used to justify the differentiation of the coastal area into the impacted and control sites according to the determined Chl-*a* level.

**Dredge spoil dumping area.** In case of the dredge spoil dumping area dataset, the information of the nMDS analysis of the macrozoobenthos abundance was used to characterise the control and impacted samples. For sites with intermediate position between control and impacted samples in the nMDS ordination plot, the earlier published density thresholds of less than 118 ind m<sup>-2</sup> and 55 ind m<sup>-2</sup> for polychaete *Pygospio elegans* and ostracods (Olenin, 1992) respectively were used to justify the impacted sites. Additionally, sampling sites with weak disturbance signals in the macrozoobenthos structure but located within the designated dredge spoil dumping area were also classified as being associated with dredge spoil dumping impacts.

**Bottom trawling area.** The nMDS analysis of the macrozoobenthos data (presence/absence) was used for the classification of samples into the impacted and control groups from the bottom trawling area. The information on the sampling site position with respect to the acoustically mapped trawling tracks was used as the criteria to justify bottom trawling effects if a low deviation from the control group of samples was observed in the ordination plot. Two different macrozoobenthos communities were characterised at 60-65 m depth interval, but due to a low number of samples they were not analysed separately.

**Accuracy range of species sensitivity values.** The accuracy range was estimated from datasets generated by the resampling of the original eutrophication, dredge spoil dumping and bottom trawling dataset using Jackknife method (Tukey, 1958). The resampling was carried out using the step-wise deletion of successive samples from the original dataset for each disturbance type separately and making new datasets each consisting of  $n-1$  samples. This procedure resulted in 64 resampled datasets for

the eutrophication area, 117 resampled datasets for the dredge spoil dumping area and 47 resampled datasets for the bottom trawling area. All these resampled datasets consisted of the control and impacted samples and were used for the calculation of alternative sensitivity values for each of species. This exercise resulted in a number of alternative species sensitivity values (one for one resampled dataset), therefore minimum and maximum values were used to define the accuracy range of sensitivity values for a given species from the dataset on the selected disturbance type.

**BQI validation and determination of water quality class threshold according to the species sensitivity groups and regression analysis.** For the coastal waters calculated species sensitivity values range was divided into 4 equal intervals following the approach of other authors (Osowiecki et al., 2008, Leonardson et al., 2009; Fleischer, Zettler, 2009). Sensitivity values intervals in the ascending order were further used to distinguish sensitivity groups as very tolerant, tolerant, sensitive and very sensitive. Water quality class boundaries (bad, poor, moderate, good, high) were determined according to the four sensitivity groups relative abundance (%) along the BQI gradient. The derived BQI threshold for the good-moderate water quality class was also compared to the good-moderate water quality class threshold estimated according to the relationship between BQI values and the summer (June-August) average of Chl-*a*. Here, the previously estimated Chl-*a* threshold for the coastal waters and plume area (Langas et al., 2009) was used to derive the BQI threshold for the good-moderate water quality class.

Testing the responsiveness of the BQI values to the eutrophication disturbance (expressed by Chl-*a*, TP, TN) a one-year lag was applied for the index values in respect to the pelagic parameters. The one-year lag was also supported by the best statistical response using the multiple linear regression ( $r=0.30$ ,  $p=0.08$ ) of the BQI values to the environmental variables compared to the no lag or two-year lag applications ( $r=0.06$ ,  $p=0.80$  and  $r=0.04$ ,  $p=0.86$  respectively). On the other hand, instant effects (no lag) were less likely to be studied due to the timing of pelagic and benthic samplings (June-August and May-September respectively).

**BQI validation and determination of water quality class threshold according to the Signal detection theory (SDT) method.** A receiver operating characteristic (ROC) curve diagram is a graphical plot that illustrates the diagnostic ability of a binary classifier system as its discrimination threshold can be selected. ROC curves consider what would happen if a particular index values were used for the response prediction analysis to the selected parameters values. Turning the index values (as a response to the selected parameter) into “yes” or “no” predictions requires to set “Gold standard” when a threshold is known. Cases with scores above the threshold are classified as positive (i.e. index sensitivity), and cases with scores below the threshold are predicted to be negative (i.e. index specificity). Different threshold values of Chl-*a*, TP, TN, defined for the Lithuanian coastal waters and plume area, were applied in the SDT analysis (Table 3).

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*Table 3.* Good-moderate water quality class threshold values for the Chl-*a*, TP, TN defined for the coastal waters and plume area (Langas et al., 2009) used in SDT analysis.

3 lentelė. Chl-*a*, bendro fosforo ir bendro azoto koncentracijų slenkstinės vertės ribai tarp geros-vidutinės vandens kokybės klasių nustatytų priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje, naudotos Signalo aptikimo teorijos metodui.

| Eutrophication parameter           | Coastal waters | Plume area |
|------------------------------------|----------------|------------|
| Chl- <i>a</i> (mg/m <sup>3</sup> ) | ≤4.8           | ≤25.7*     |
| TP (μg/l)                          | ≤26            | ≤26**      |
| TN (μg/l)                          | ≤250           | ≤250**     |

\*-at salinities <4

\*\* -at salinities >4

The graphical visualizations of the BQI values response to the selected parameters (Chl-*a*, TP, TN) and threshold values determination (Chl-*a*) for the good-moderate water quality class boundaries analysis were performed in the R v3 statistical computing environment (R-project., 2014, unpublished R-script). ROC curves were used as a visual tool for assessing the accuracy of BQI index to the selected parameters (Chl-*a*, TP, TN), by plotting the probability of the true positives (sensitivity) against the probability of the true negatives (specificity). Index sensitivity and specificity values can be estimated using formulas (Murtaugh, 1996):

$$\text{Sensitivity value} = \frac{\text{True positives}}{(\text{true positives} + \text{false negatives})} \quad (10)$$

$$\text{Specificity value} = \frac{\text{True negatives}}{(\text{false positives} + \text{true negatives})} \quad (11)$$

The area under the ROC curve (AUC) was used as a measure of the BQI response. A perfect index results in AUC value of 1, whereas 0.5 is a result of a non-informative index (Murtaugh, 1996). In this study the interpretation of AUC values followed the rules described in Hale and Heltshe (2008), which define  $0.7 \leq \text{AUC}$  and  $\text{AUC} \geq 0.8$  as indicating acceptable and excellent responses, respectively.

Due to the reliable satellite data of the surface water Chl-*a*, the BQI values response to the Chl-*a* was used to set the good-moderate water quality class boundaries. The determinate BQI values thresholds were assigned at different index specificity and sensitivity levels. The predictive ability of the BQI values 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). To

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calculate PPV and NPV values, the environment condition prevalence at the analysed study area or sampling site (in this case the number of samples having the good-moderate water quality status of Chl-*a*) was calculated (Swets et al., 2000):

$$\frac{\text{Number of samples in good – moderate water quality status}}{\text{Number of all studied samples}} \quad (12)$$

Positive predictive value and negative predictive value were estimated using formulas (Murtaugh, 1996):

$$PPV = \frac{(Sensitivity * prevalence)}{[(Sensitivity * prevalence) + (1 - Specificity) * (1 - prevalence)]} \quad (13)$$

$$NPV = \frac{Specificity * (1 - prevalence)}{[(Specificity) * (1 - prevalence) + (1 - Sensitivity) * prevalence]} \quad (14)$$

The PPV and NPV values vary, according to the prevalence of the target values in the analysed parameter, i.e. values at or above the good water quality threshold for this study. For example, at low prevalence of the target values a correct (true positive) response will only be attained with an accurate index, implying a low rate of false positives (Swets et al., 2000). Thus, by using PPV and NPV, the probability of getting a correct response is evaluated against the risks of making wrong decisions. Such an approach was also used in this study to set the index thresholds for distinguishing the impacted samples from the undisturbed ones.



# 4

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## Results

### 4.1 Macrozoobenthos characteristics along the depth gradient

**Macrozoobenthos composition.** In total 24 soft bottom macrozoobenthos species or higher order taxa were recorded in the study area, while due to the reliable number of samples where species occur at every compiled dataset 16 taxa were used for analysis. Crustaceans, polychaetes and bivalves were the most diverse taxonomic groups comprising 42%, 21% and 17% of total recorded taxa respectively.

The analysis of macrozoobenthos species composition along the depth gradient from 10 to 70 m depth showed three groups of species (Table 4). The group of shallow taxa consists of 7 species, half of them were distributed down to 20-30 m depth, while other continued to colonise depths down to 40 m. Bivalves *Cerastoderma glaucum*, *Mya arenaria* and polychaete *Hediste diversicolor* had one of the highest occurrences among these shallow species, however the occurrence of all species belonging to this group decreased with the depth and particularly in the depth interval of 30-40 m (Table 4).

The second group of 4 species or higher order taxa (oligochaetes, polychaetes *P. elegans*, *Marenzelleria* spp. and bivalve *L. balthica*) was the most widespread along the analysed depth gradient. All these taxa were present down to 70 m depth, but *L. balthica* and *Marenzelleria* spp. had extremely wide distribution, the occurrence of this group of organisms within the depth intervals rarely dropped below 70-100% (Table 4).

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The third group of deeper water organisms (polychaete *Bylgides sarsi*, priapulid *H. spinulosus*, amphipod *Monoporeia affinis*, isopod *Saduria entomon* and ostracods) were distributed in depths higher than 30 m. In many cases (except for *S. entomon*) the occurrence of these species highly varied irrespective of the depth (Table 4).

*Table 4.* Characterisation and grouping of the macrozoobenthos species according to their distribution depth limits at the 10-70 depth range. Species occurrence is indicated for a given depth interval within the corresponding cell. See page 41 in chapter 3.2.3 for calculation of species distribution upper/lower boundaries. Black frames indicate three groups of species (shallow species, widespread species, deep species).

4 lentelė. Makrozoobentoso rūšių grupavimas pagal jų sutinkamumą, viršutines ir apatines rūšių paplitimo ribas bei rūšių skaičių, 10-70 m gylyje. Juoduose rėmuose apibrauktos išskirtos makrozoobentoso grupės (sekliamėgės, giliamėgės, paplitusios visame gylyje).

| Species/taxa<br>Depth range (m)                    | Species<br>group name | 10-20 | 20-30 | 30-40 | 40-50 | 50-60 | 60-70 |
|----------------------------------------------------|-----------------------|-------|-------|-------|-------|-------|-------|
| <i>Streblospio shrubsolii</i>                      | shallow               | 47    |       |       |       |       |       |
| <i>Bathyporeia pilosa</i>                          | shallow               | 29    |       |       |       |       |       |
| <i>Cerastoderma glaucum</i>                        | shallow               | 71    | 80    | 40    |       |       |       |
| <i>Hydrobia sp.</i>                                | shallow               | 74    | 60    | 53    |       |       |       |
| <i>Mya arenaria</i>                                | shallow               | 91    | 80    | 63    |       |       |       |
| <i>Corophium volutator</i>                         | shallow               | 50    | 87    | 57    |       |       |       |
| <i>Hediste diversicolor</i>                        | shallow               | 100   | 100   | 80    |       |       |       |
| <i>Oligochaeta undet.</i>                          | widespread            | 97    | 87    | 97    | 90    | 41    | 15    |
| <i>Pygospio elegans</i>                            | widespread            | 91    | 73    | 100   | 95    | 93    | 92    |
| <i>Marenzelleria spp.</i>                          | widespread            | 97    | 100   | 100   | 100   | 100   | 100   |
| <i>Limecola balthica</i>                           | widespread            | 100   | 100   | 100   | 100   | 100   | 100   |
| <i>Bylgides sarsi</i>                              | deep                  |       |       | 73    | 60    | 30    | 69    |
| <i>Halicryptus spinulosus</i>                      | deep                  |       |       | 93    | 100   | 59    | 69    |
| <i>Monoporeia affinis</i>                          | deep                  |       |       | 27    | 45    | 63    | 77    |
| <i>Ostracoda undet.</i>                            | deep                  |       |       | 17    | 56    | 63    | 44    |
| <i>Saduria entomon</i>                             | deep                  |       |       | 87    | 95    | 100   | 100   |
| Number of samples                                  |                       | 34    | 15    | 30    | 20    | 27    | 13    |
| Number of species upper<br>distribution boundaries |                       | 0     | 0     | 5     | 0     | 0     | 0     |
| Number of species lower<br>distribution boundaries |                       | 2     | 0     | 5     | 0     | 0     | 0     |
| Number of species                                  |                       | 11    | 9     | 13    | 9     | 9     | 9     |

The depth range of 30-40 m can be distinguished as having the highest number of species distribution boundaries along the analysed depth gradient (Table 4). This depth is being occupied by majority of the recorded species in the study but the occur-



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rence of shallow taxa here is considerably reduced. Five species (*C. glaucum*, *Hydrobia* sp., *M. arenaria*, *Corophium volutator*, *H. diversicolor* species) had the deepest occurrence (lower distribution boundary) in this depth and another 5 deeper living species (*B. sarsi*, *H. spinulosus*, *M. affinis*, *Ostracoda* undet., *S. entomon*) were recorded at their most shallow sites (upper distribution boundary).

**Macrozoobenthos abundance.** The nMDS analysis of square root transformed abundance data showed the most meaningful grouping of samples taken at three depth intervals (10-30 m, 30-50 m, 50-70 m) (Fig. 6). The nMDS analysis grouping reflects relatively continuous gradient of macrozoobenthos abundance change along the depth intervals. The largest overlap of samples is found between two shallow (10-20 m and 20-30 m) and between two deepest (50-60 and 60-70 m) depth intervals, while samples from two other depth ranges (30-40 and 40-50 m depth) form relatively distinct intermediate groups between shallow and deeper samples.

According to the SIMPER analysis, similarity in the 10-30 m depth interval (10-20 m depth interval 42.68% and 20-30 m depth interval 36.46%) and 50-70 m (50-60 m depth interval 50.31% and 60-70 m depth interval 41.96%) was bigger than in 30-50 m depth interval (30-40 m depth interval 45.17% and 40-50 m depth interval - 40.51% respectively). At the same time, SIMPER analysis showed high dissimilarity (66.78%) between samples of 20-30 m and 30-40 m with spionids *Marenzelleria* spp. and *P. elegans* contributing a half of this difference (24.4% and 13.2% respectively). Relatively high dissimilarity of 59.33% was also found between the groups of samples from depths of 40-50 m and 50-60 m, where an important role of spionids (38.9% *Marenzelleria* spp. and 16.0% *P. elegans*) for the differentiation of the groups was complemented by contribution of *L. balthica* (14.1%). These findings supported decision to merge intermediate depth intervals of 30-40 m and 40-50 m into one group due to practical reasons of having the larger sample size for a group for comparative analysis. Merging of 30-50 m samples into one group was also consistent with classification results according to the abundance data (Fig. 5), where both 30-40 and 40-50 m depth samples formed distinct intermediate groups between shallow and deeper sites. The differences in the macrozoobenthos structure based on the nMDS grouping were tested using one-way analysis of similarities test (ANOSIM). According to the analysis results, the difference of 30-50 m depth range from 10-30 and 50-70 m was similar and of marginal significance ( $R=0.53$ ,  $p=0.1$  and  $R=0.41$ ,  $p=0.1$  respectively).

Within the depth interval of 10-30 m total macrozoobenthos abundance varied between 264 and 18 990 ind m<sup>-2</sup> (average  $4870 \pm 3742$  ind m<sup>-2</sup>). Soft-shell clam species *C. glaucum* and *M. arenaria*, crustacean *C. volutator*, polychaete *H. diversicolor* composed the pool of the most abundant shallow species. In contrast, the average abundance of a typical shallow amphipod *Bathyporeia pilosa* was very low ( $7 \pm 24$  ind m<sup>-2</sup>).

In depths between 30-50 m, the total average of macrozoobenthos abundance was  $3445 \pm 2667$  ind m<sup>-2</sup>. Compared to the coastal area (10-30 m depth), typical shallow

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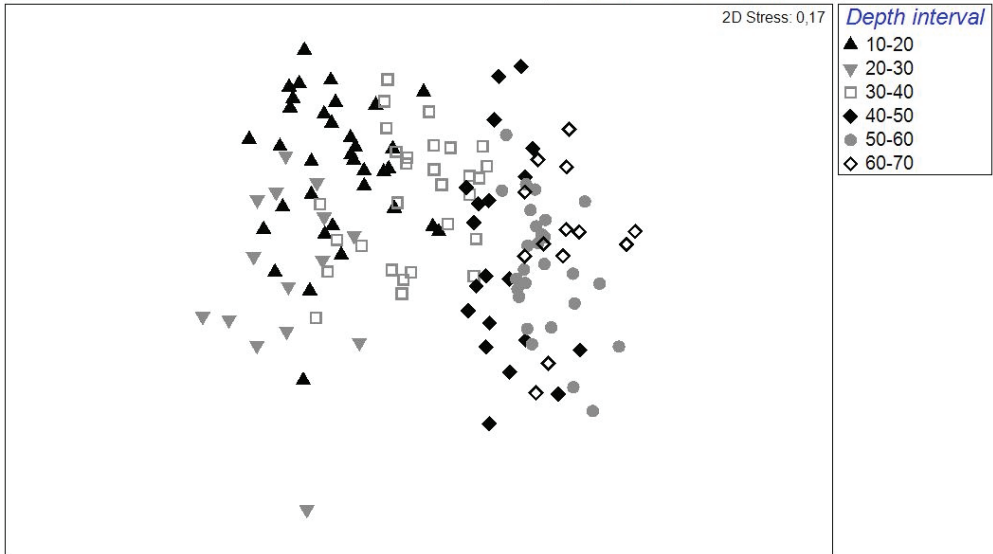


Figure 5. nMDS plot representing classification of macrozoobenthos samples according to square root species abundance in different depths.

5 paveikslas. Makrozoobentos gausumo mėginių, surinktų iš skirtingų gylių, klasifikacija pagal nMDS analizę.

species *M. arenaria* and *C. volutator* reduced their abundance and occurrence in this depth zone down to  $85 \pm 436$  ind  $m^{-2}$  and less than 30% respectively. Glacial relict species were recorded in high occurrence-isopod *S. entomon* (average abundance  $56 \pm 173$  ind  $m^{-2}$ , occurrence 90%), ostracods ( $19 \pm 61$  ind  $m^{-2}$ , 42%) and *M. affinis* ( $130 \pm 504$  ind  $m^{-2}$ , 34%). Along with the permanently present *L. balthica*, the highest occurrence was also characteristic for spionids *Marenzelleria* spp. (100%) and *P. elegans* (98%).

In depth range of 50-70 m, the total average of macrozoobenthos abundance  $1734 \pm 1181$  ind  $m^{-2}$ . The pool of characteristic species was formed by polychaetes *P. elegans* (average  $302 \pm 316$  ind  $m^{-2}$ , occurrence 93%), *Marenzelleria* spp. (average  $761 \pm 788$  ind  $m^{-2}$ , occurrence 100%), *B. sarsi* (average  $9 \pm 14$  ind  $m^{-2}$ , occurrence 43%), priapulid *H. spinulosus* (average  $28 \pm 38$  ind  $m^{-2}$ , occurrence 63%) ostracods (average  $93 \pm 163$  ind  $m^{-2}$ , occurrence 63%) and isopod *S. entomon* (average  $51 \pm 95$  ind  $m^{-2}$ , occurrence 100%).

Summarizing the results above, it is obvious that in spite of several widespread species common along the entire depth gradient, 10-30 and 50-70 m depth intervals form a relatively distinct macrozoobenthos groups, different in terms of taxonomic composition and abundance. Major taxonomic shift in macrozoobenthos structure within 30-40 m depth overlaps with the largest abundance changes at 30-50 m depth, therefore the studied depth gradient is divided into shallow (down to 30 m), deep (be-

low 50 m) and intermediate depths (30-50 m) for further analysis of natural variability of species sensitivity values.

### 4.2 Reliability of species sensitivity values

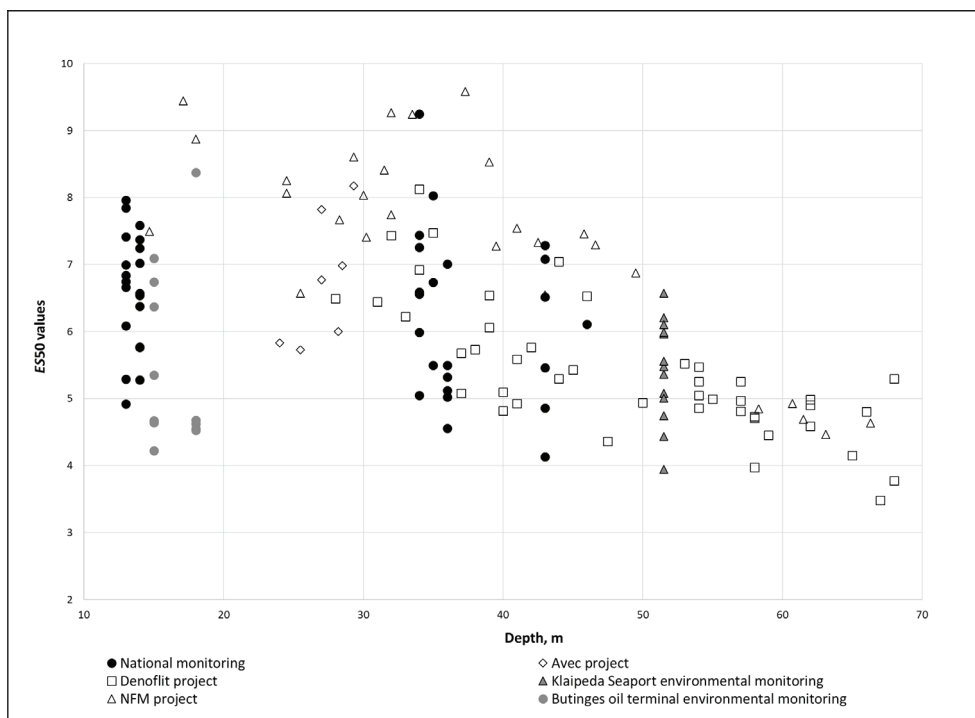
#### 4.2.1 Species sensitivity values changes along the depth gradient

In order to assess the role of depth in the variability of the species sensitivity values, the analysis was performed on samples grouped into three depth intervals (10-30 m, 30-50 m, 50-70 m) justified above (see page 47 in chapter 4.1). The variation of calculated *ES50* values showed considerable differences between depth intervals, but not between data of different origin and sampling periods (Fig. 6). Data for all depths were provided by more than one sampling programme, however repeated sampling (supported by monitoring programmes only) covered depths down to 52 m only.

The estimated *ES50* values ( $n=139$ ) varied from 3.5 to 9.6 along the entire analysed depth range (10-70 m) (Fig. 6). At the 10-30 m depth interval, *ES50* values were the highest and values less than 5.5 were characteristic only for 22% of all analysed values. In contrast, at the 50-70 m depth interval, *ES50* values less than 5.5 were estimated for 85% of all analysed samples. Statistically significant difference in *ES50* values were found between all three (10-30 m, 30-50 m and 50-70 m) depth intervals (Kruskal-Wallis, Chi-square=37.98,  $p=0.0001$ ).

There was no single species with stable sensitivity values along the studied depth gradient. On the other hand, only 4 out of 16 analysed macrozoobenthos taxa were present along the entire depth range. These lowest sensitivity values taxa, spionids *Marenzelleria* spp., *P. elegans*, oligochaetes and bivalve *L. balthica* had relatively constant sensitivity values at depths down to 50 m, where their abundance and occurrence were the highest (Table 5). Deeper than 50 m sensitivity values of these taxa decreased with decreasing abundance, although all of them (except oligochaetes) remained among the most abundant in macrozoobenthos. In contrast, sensitivity values of shallow species *M. arenaria*, *C. glaucum*, polychaete *H. diversicolor* and crustacean *C. volutator* increased from 10-30 m to 30-50 m depth range at decreasing abundance. Two other shallow species, amphipod *B. pilosa* and polychaete *Streblospio shrubsolii* were found only in 10-30 m depth interval in low abundance and occurrence. Group of deeper-living taxa (ostracods, *H. spinulosus*, *M. affinis*, *B. sarsi*, *S. entomon*) typically had decreasing sensitivity values with increasing depth (from 30-50 to 50-70 m) and decreasing abundance (Table 5). In 50-70 m depth interval species sensitivities were calculated for nine macrozoobenthos species, the lowest values were found for polychaetes *Marenzelleria* spp., and *B. sarsi*, while the highest were recorded for *Ostracoda* undet. and amphipod *M. affinis* (Table 5).

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*Figure 6.* Variability of ES50 values along the depth range from 10 to 70 m based on samples classification according to their origin. Different symbols show different sampling programmes/periods (see section 4.2.1 for description) with solid symbols indicating repeated sampling from long-term monitoring programmes and open symbols denoting data from project based samplings with no temporal repetition.

*6 paveikslas.* ES50 verčių dinamika 10-70 m gylio intervale. Skirtingi simboliai nurodo skirtingas mėginių rinkimo programas ir laikotarpius: juodi, užpildyti simboliai žymi pagal ilgalaikes monitoringo programas rinktus mėginius, šviesūs, neužpildyti simboliai – trumpalaikių projektų veiklose rinktus mėginius.

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**Table 5. Macrozoobenthos species sensitivity values calculated for different datasets of the depth intervals.**

**5 lentelė. Makrozoobentoso rūšių jautrumo vertės apskaičiuotos skirtingiems duomenų rinkiniams iš skirtingų gylio intervalų.**

| Macrozoobenthos species/taxa  | Species sensitivity values<br>average abundance (ind m <sup>-2</sup> )<br>number of samples |                                   |                                 |                                    |
|-------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------|---------------------------------|------------------------------------|
|                               | 10-30 m                                                                                     | 30-50 m                           | 50-70 m                         | 10-70 m                            |
| <i>Marenzelleria</i> spp.     | <b>4.6</b><br>(1556±1696)<br>(48)                                                           | <b>4.6</b><br>(1265±1148)<br>(50) | <b>3.5</b><br>(761±788)<br>(40) | <b>4.1</b><br>(1223±1324)<br>(138) |
| <i>Pygospio elegans</i>       | <b>4.2</b><br>(548±738)<br>(42)                                                             | <b>4.6</b><br>(507±546)<br>(49)   | <b>3.9</b><br>(302±316)<br>(37) | <b>4.1</b><br>(462±580)<br>(128)   |
| <i>Limecola balthica</i>      | <b>5.3</b><br>(366±384)<br>(49)                                                             | <b>5.0</b><br>(450±510)<br>(50)   | <b>3.8</b><br>(404±337)<br>(40) | <b>4.1</b><br>(409±421)<br>(139)   |
| <i>Oligochaeta</i> undet.     | <b>4.9</b><br>(847±1489)<br>(46)                                                            | <b>4.6</b><br>(547±659)<br>(47)   | <b>4.0</b><br>(8±17)<br>(14)    | <b>4.9</b><br>(501±1022)<br>(107)  |
| <i>Mya arenaria</i>           | <b>5.3</b><br>(475±653)<br>(43)                                                             | <b>5.5</b><br>(31±55)<br>(24)     | -                               | <b>5.3</b><br>(178±446)<br>(67)    |
| <i>Hydrobia</i> sp.           | <b>6.5</b><br>(262±517)<br>(34)                                                             | <b>6.4</b><br>(56±111)<br>(16)    | -                               | <b>6.4</b><br>(113±28)<br>(50)     |
| <i>Hediste diversicolor</i>   | <b>4.9</b><br>(498±517)<br>(49)                                                             | <b>6.4</b><br>(132±243)<br>(27)   | -                               | <b>5.3</b><br>(113±333)<br>(76)    |
| <i>Corophium volutator</i>    | <b>5.7</b><br>(151±447)<br>(30)                                                             | <b>6.6</b><br>(140±612)<br>(19)   | -                               | <b>5.7</b><br>(103±456)<br>(49)    |
| <i>Cerastoderma glaucum</i>   | <b>5.7</b><br>(76±102)<br>(36)                                                              | <b>6.9</b><br>(7±16)<br>(13)      | -                               | <b>5.7</b><br>(29±70)<br>(49)      |
| <i>Bathyporeia pilosa</i>     | <b>5.3</b><br>(7±24)<br>(10)                                                                | -                                 | -                               | <b>5.3</b><br>(3±14)<br>(10)       |
| <i>Streblospio shrubsolii</i> | <b>5.8</b><br>(68±160)<br>(16)                                                              | -                                 | -                               | <b>5.8</b><br>(24±100)<br>(16)     |

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| Macrozoobenthos species/taxa  | Species sensitivity values<br>average abundance (ind m <sup>-2</sup> )<br>number of samples |                          |                         |                         |
|-------------------------------|---------------------------------------------------------------------------------------------|--------------------------|-------------------------|-------------------------|
|                               | 10-30 m                                                                                     | 30-50 m                  | 50-70 m                 | 10-70 m                 |
| <i>Ostracoda</i> undet.       | -                                                                                           | 5.3<br>(19±61)<br>(21)   | 4.6<br>(93±163)<br>(25) | 4.6<br>(34±9)<br>(46)   |
| <i>Halicryptus spinulosus</i> | -                                                                                           | 4.3<br>(80±82)<br>(48)   | 3.8<br>(28±38)<br>(25)  | 4.7<br>(39±66)<br>(73)  |
| <i>Monoporeia affinis</i>     | -                                                                                           | 5.4<br>(130±504)<br>(17) | 4.5<br>(77±150)<br>(27) | 4.5<br>(69±317)<br>(44) |
| <i>Bylgides sarsi</i>         | -                                                                                           | 5.3<br>(24±26)<br>(34)   | 3.5<br>(9±14)<br>(17)   | 4.7<br>(14±22)<br>(51)  |
| <i>Saduria entomon</i>        | -                                                                                           | 5.4<br>(56±173)<br>(45)  | 4.0<br>(51±95)<br>(40)  | 4.5<br>(35±118)<br>(85) |

#### 4.2.2 Changes of species sensitivity values between macrozoobenthos communities

Sensitivity values were calculated for the species of two different macrozoobenthos communities in the coastal waters (10-30 m depth) dominated by i) bivalve *L. balthica*, ii) polychaete *Marenzelleria* spp. Additionally, the calculation was carried out for pooled data from both communities together (Table 6). Sensitivity values for pooled data served as a reference for analysis, whether sensitivity values estimated irrespective of community type will deviate considerably from community specific sensitivity value for the same species.

Altogether 16 soft bottom macrozoobenthos species were found in *L. balthica* community with the species number in samples varying between 5 and 11, while *Marenzelleria* spp. dominated community consisted of 13 species with 2 to 9 species per sample. Three macrozoobenthos species, isopod *S. entomon*, polychaete *B. sarsi* and *S. shrubsolii* were not present in *Marenzelleria* spp. community.

The abundance of all species (except polychaete *P. elegans*) in the *Marenzelleria* spp. community was lower compared to their abundance in the *L. balthica* community by the order of magnitude and the average macrozoobenthos abundance between the communities was significantly different ( $t=1.54$ ,  $p=0.0001$ ;  $2002\pm6250$  ind m<sup>-2</sup> and  $4216\pm3707$  ind m<sup>-2</sup> respectively) (Table 6). The biomass dominance of *Marenzelleria* spp. was achieved through the decrease of *L. balthica* abundance rather than the increase of this spionid abundance (Table 6).

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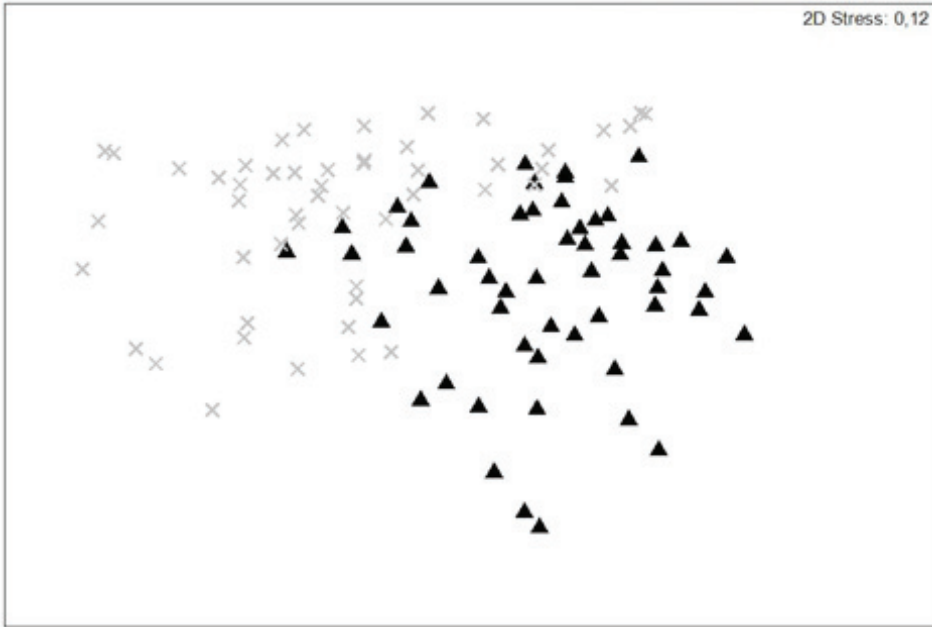


Figure 7. nMDS plot based on not transformed abundance data for two macrozoobenthos communities: x – polychaete *Marenzelleria* spp. community, ▲ – bivalve *L. balthica* community.

7 paveikslas. Makrozoobentos mėginių, surinktų iš skirtingų bendrijų, klasifikacija pagal gausumo nMDS analizę. x – daugiašerės kirmėlės *Marenzelleria* spp. bendrija, ▲ – dvigeldžio moliusko *L. balthica* bendrija.

The analysis of not transformed abundance data by nMDS resulted in clear discrimination of samples that coincides with the changes in biomass dominant species and leaves negligible overlap from two communities in the ordination plot (Fig. 7).

Macrozoobenthos species sensitivity values showed significant difference (Mann-Whitney test,  $p=0.0001$ ,  $z=-4.512$ ) between the analysed communities with 4.1-5.2 range of species sensitivity values for *L. balthica* community, 1.9-3.9 range for *Marenzelleria* spp. community) (Table 6). In both communities, polychaete *Marenzelleria* spp. was the most abundant taxa and had the lowest sensitivity values (4.1 in the *L. balthica* community and 1.9 in the *Marenzelleria* community). On the other hand, there was no pattern observed between change in the species abundance and its sensitivity value in two different communities. For example, polychaete *H. diversicolor* had one of the highest sensitivity values (5.2) in the *L. balthica* community, but one of the lowest sensitivity values (1.9) in *Marenzelleria* spp. community at nearly 10 times lower abundance. In contrast, bivalves *L. balthica* and *M. arenaria* maintained

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high sensitivity values in both communities under similar density decrease as in case of *H. diversicolor*. Moreover, sensitivity values changed considerably from 4.5 to 3.0 for polychaete *P. elegans* that was the only species with the comparable average abundance in both analysed communities (Table 6).

**Table 6.** Species sensitivity values estimated for samples from *L. balthica*, *Marenzelleria* spp. communities and for pooled data from both communities in the coastal waters (10-30 m depth).

**6 lentelė.** Rūšių jautrumo vertės, paskaičiuotos dvigeldžio moliusko *L. balthica*, daugiašerės kirmėlės *Marenzelleria* spp. bendrijoms ir bendram abiejų bendrijų duomenų rinkiniui priekrantėje (10-30 m gylyje).

| Macrozoobenthos species/taxa | Species sensitivity values, abundance (ind m <sup>-2</sup> ), number of samples |                                     |                                |
|------------------------------|---------------------------------------------------------------------------------|-------------------------------------|--------------------------------|
|                              | <i>L. balthica</i> community                                                    | <i>Marenzelleria</i> spp. community | Both communities (pooled data) |
| <i>Marenzelleria</i> spp.    | 4.1<br>(1435±1567)<br>(54)                                                      | 1.9<br>(1369±4767)<br>(51)          | 2.1<br>(1402±3532)<br>(105)    |
| <i>Pygospio elegans</i>      | 4.5<br>(438±615)<br>(48)                                                        | 3.0<br>(452±2001)<br>(39)           | 3.0<br>(445±1473)<br>(87)      |
| <i>Oligochaeta</i> undet.    | 5.2<br>(736±1437)<br>(51)                                                       | 2.8<br>(50±106)<br>(35)             | 4.7<br>(393±1071)<br>(86)      |
| <i>Limecola balthica</i>     | 4.9<br>(377±424)<br>(54)                                                        | 3.0<br>(29±104)<br>(17)             | 3.1<br>(203±353)<br>(71)       |
| <i>Mya arenaria</i>          | 4.9<br>(364±628)<br>(42)                                                        | 3.9<br>(40±110)<br>(15)             | 4.6<br>(202±477)<br>(57)       |
| <i>Hediste diversicolor</i>  | 5.2<br>(410±498)<br>(54)                                                        | 1.9<br>(43±52)<br>(35)              | 3.8<br>(227±398)<br>(89)       |

Assessing species sensitivity values irrespective of community (pooled data) three major patterns were observed in changes of macrozoobenthos species sensitivity values and abundance. First, polychaetes *Marenzelleria* spp., *P. elegans* and bivalve *L. balthica* had similar sensitivity values and average abundance (except for *L. balthica*) between pooled data and *Marenzelleria* spp. community dataset (Table 6). In contrast, sensitivity values of oligochets and *M. arenaria* were similar in pooled dataset and *L. balthica* community. *H. diversicolor* had the highest difference of sensitivity values between two communities, but the pooled dataset delivered approximately averaged sensitivity and abundance values (Table 6). Overall, this analysis demonstrates a con-



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siderable deviation of community-specific species sensitivity values from pooled data estimates for all the analysed taxa. Although few species may retain sensitivity values when moving from general dataset to the specific community, but this values typically gets innaccurate when applying it to the conditions of another specific community.

### 4.2.3 The role of macrozoobenthos community disturbance in the species sensitivity values assessment

#### 4.2.3.1 Species sensitivity values under eutrophication effects

In total 12 soft bottom macrozoobenthos species were recorded from 65 sites in depths of 10-20 m. According to the nMDS analysis performed on not transformed abundance data 25 sites were classified as impacted and 40 sites assigned to the control group (Fig. 8).

At the control sites macrozoobenthos richness varied from 6 to 10 species and total average of abundance was similar as at the impacted sites ( $7306 \pm 4695$  ind  $m^{-2}$  and  $7586 \pm 9641$  ind  $m^{-2}$  respectively; Mann-Whitney test,  $W=145$ ,  $p=0.086$ ), where species number varied from 2 to 10.

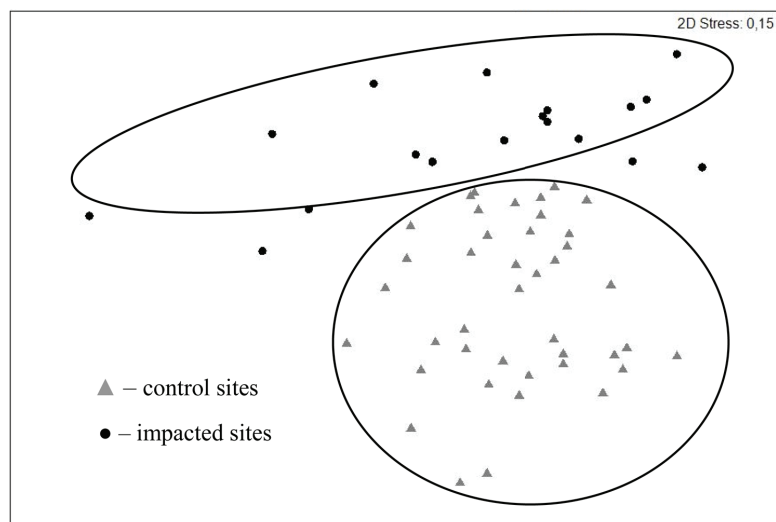


Figure 8. nMDS plot for classification of control and eutrophication impacted sites according to macrozoobenthos abundance in the coastal waters.

*8 paveikslas.* Eutrofikacijos poveikio ir foninių mėginių nMDS analizės klasifikacija pagal makrozoobentos gausumą priekrantėje.

#### 4. Results

Species sensitivity values were calculated for all 12 species using samples from the control area only and employing a full dataset representing both impact and control sites (Table 7). Sensitivity values and their range were broader in full dataset compared to the control dataset (2.3-5.8 and 4.3-5.8, respectively).

More than half of the species had significantly lower sensitivity values calculated from full dataset (including impacted sites) than from the control sites (Wilcoxon test,  $p=0.0180$ ,  $z=-2.371$ ). After changing datasets and including the impacted sites, the largest decrease in sensitivity values was detected for spionids *Marenzelleria* spp. and *P. elegans*, polychaete *H. diversicolor*, bivalve *L. balthica* and crustacean *C. volutator*, while relatively minor changes were found for bivalve *M. arenaria* (Table 7).

The sensitivity values did not change between the datasets for five species or higher order taxa-clam *C. glaucum*, *Nemertini* undet., polychaete *S. shrubsolii*, amphipod *B. pilosa* and bivalve *Hydrobia* sp. The sensitivity values of 6 species from the control sites fall outside the accuracy range derived from the full dataset (Table 7).

**Table 7. Comparison of species sensitivity values based on control sites dataset and full dataset (including samples with elevated Chl-*a*).  
Accuracy range of the species sensitivity values.**

**7 lentelė. Rūšių jautrumo verčių, skaičiuotų foninių vietų duomenų rinkiniui ir bendram foninių ir poveikio mėginių duomenų rinkiniui (įtraukiant mėginius su didele Chl-*a* koncentracija), palyginimas. Paskaičiuotas rūšių jautrumo verčių tikslumo intervalas.**

| Macrozoobenthos species/<br>taxa | Control dataset            |                   |                                          | Full dataset               |                   |                                          |                                              |
|----------------------------------|----------------------------|-------------------|------------------------------------------|----------------------------|-------------------|------------------------------------------|----------------------------------------------|
|                                  | Species sensitivity values | Number of samples | Species abundance (ind m <sup>-2</sup> ) | Species sensitivity values | Number of samples | Species abundance (ind m <sup>-2</sup> ) | Accuracy range of species sensitivity values |
| <i>Marenzelleria</i> spp.        | 5.3*                       | 37                | 1369±1841                                | 2.3                        | 65                | 3204±6023                                | 2.3                                          |
| <i>Pygospio elegans</i>          | 4.3*                       | 39                | 1753±2895                                | 2.5                        | 62                | 1485±2816                                | 2.5                                          |
| <i>Limecola balthica</i>         | 4.4*                       | 38                | 560±578                                  | 3.1                        | 64                | 402±506                                  | 3.1-3.2                                      |
| <i>Oligochaeta</i> undet.        | 4.8*                       | 40                | 1335±1666                                | 4.4                        | 64                | 822±1393                                 | 4.4-4.6                                      |
| <i>Corophium volutator</i>       | 5.3*                       | 13                | 8±25                                     | 2.3                        | 31                | 20±46                                    | 2.3                                          |
| <i>Hediste diversicolor</i>      | 5.2*                       | 40                | 405±476                                  | 3.8                        | 65                | 324±405                                  | 3.6-3.8                                      |
| <i>Mya arenaria</i>              | 4.6                        | 38                | 1188±1566                                | 4.4                        | 60                | 741±1295                                 | 4.4-4.6                                      |

## 4. Results

| Macrozoobenthos species/taxa  | Control dataset            |                   |                                          | Full dataset               |                   |                                          |                                              |
|-------------------------------|----------------------------|-------------------|------------------------------------------|----------------------------|-------------------|------------------------------------------|----------------------------------------------|
|                               | Species sensitivity values | Number of samples | Species abundance (ind m <sup>-2</sup> ) | Species sensitivity values | Number of samples | Species abundance (ind m <sup>-2</sup> ) | Accuracy range of species sensitivity values |
| <i>Bathyporeia pilosa</i>     | 5.3                        | 17                | 13±30                                    | 5.3                        | 17                | 7±24                                     | 5.3-5.4                                      |
| <i>Nemertini undet.</i>       | 4.3                        | 11                | 5±10                                     | 4.3                        | 11                | 3±8                                      | 4.3-4.8                                      |
| <i>Cerastoderma glaucum</i>   | 4.8                        | 33                | 65±82                                    | 4.8                        | 40                | 42±69                                    | 4.6-4.9                                      |
| <i>Streblospio shrubsolii</i> | 5.8                        | 9                 | 67±174                                   | 5.8                        | 17                | 46±137                                   | 5.8-6.3                                      |
| <i>Hydrobia</i> sp.           | 4.4                        | 29                | 538±1043                                 | 4.4                        | 43                | 330±826                                  | 4.4-4.6                                      |

\*-species sensitivity values outside the accuracy range derived from full dataset.

### 4.2.3.2 Species sensitivity values under dredge spoil dumping effects

Altogether 16 soft bottom macrozoobenthos species were recorded in the *L. balthica* community in the dredge spoil dumping area within the depth range of 40-50 m. Next to this dominant bivalve (relative biomass higher than 40%), spionids *Marenzelleria* spp. and *P. elegans* as well as isopod *S. entomon* were characteristic taxa in the community in terms of their high occurrence (89-90%) and relative biomass (2%, 1% and 14% respectively).

According to the nMDS analysis performed on not transformed abundance data 37 sites were classified as impacted and eighty-one sites assigned to the control group (Fig. 9). Dredge spoil dumping effects were characterized by the absence or significant reduction of average abundance of spionid *P. elegans* from 511±424 ind m<sup>-2</sup> in the control sites to 36±37 ind m<sup>-2</sup> in the impacted sites (Mann-Whitney test, W=-1498.5, p<0.0001) (Fig. 10). Additionally, the control and impacted sites differ significantly (t=5.54, p=0.0001) in terms of the total average macrozoobenthos abundance (1376±747 ind m<sup>-2</sup> and 630±506 ind m<sup>-2</sup> respectively) and species richness which varied from 5 to 12 at control sites and between 2 and 9 at impacted sites.

The sensitivity values were calculated for 8 species using samples from the control sites only and employing a full dataset representing both impact and control sites (Table 8). Although the range of sensitivity values obtained from both datasets was similar (4.1-4.9 for the control sites, and 3.7-4.8 for the full dataset), the sensitivity values of 5 species from the control sites fall outside the accuracy range derived from the full dataset.

#### 4. Results

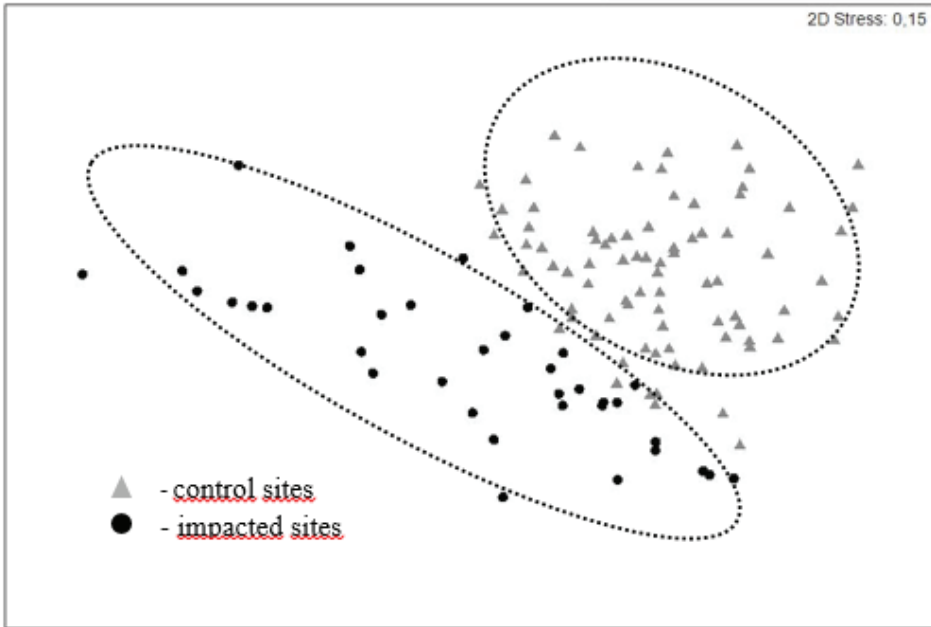


Figure 9. nMDS plot for classification of control and impacted sites within the dredge spoil dumping area according to macrozoobenthos abundance.

9 paveikslas. Grunto pylimo poveikio ir foninių mėginių nMDS analizės klasifikacija pagal makrozoobentosos gausumą.

After changing the datasets and including the impacted samples into analysis, the largest decrease in sensitivity values was detected for isopod *S. entomon* and bivalve *L. balthica*, while relatively minor changes were found for *B. sarsi* and *H. spinulosus*. The sensitivity values for *P. elegans* and ostracods did not change between the used datasets (Table 8). Overall, the accuracy range estimated for the sensitivity values of individual taxa indicated a very high stability of values (not exceeding 10% change from the original value) for all the species except for *B. sarsi* (Table 8). Although the latter had the lowest abundance and the lowest number of samples, no clear dependency between these two parameters and sensitivity values range was observed for the rest of the species.

## 4. Results

**Table 8.** Comparison of species sensitivity values based on control sites dataset and full dataset (including samples impacted by dredge spoil dumping effect). Accuracy range of the species sensitivity values.

**8 lentelė.** Rūšių jautrumo verčių, skaičiuotų foninių vietų duomenų rinkiniui ir bendram foninių ir poveikio mėginių duomenų rinkiniui (įtraukiant mėginius su grunto pylimo poveikiu), palyginimas. Paskaičiuotas rūšių jautrumo verčių tikslumo intervalas.

| Macrozoobenthos species/<br>taxa | Control dataset            |                   |                                          | Full dataset               |                   |                                          |                                              |
|----------------------------------|----------------------------|-------------------|------------------------------------------|----------------------------|-------------------|------------------------------------------|----------------------------------------------|
|                                  | Species sensitivity values | Number of samples | Species abundance (ind m <sup>-2</sup> ) | Species sensitivity values | Number of samples | Species abundance (ind m <sup>-2</sup> ) | Accuracy range of species sensitivity values |
| <i>Marenzelleria</i> spp.        | 4.1                        | 80                | 338±331                                  | 3.9                        | 108               | 312±344                                  | 3.7-4.1                                      |
| <i>Pygospio elegans</i>          | 4.1                        | 81                | 511±424                                  | 4.1                        | 106               | 362±415                                  | 4.1                                          |
| <i>Ostracoda</i> undet.          | 4.5                        | 51                | 28±41                                    | 4.5                        | 66                | 26±47                                    | 4.5-4.8                                      |
| <i>Limecola balthica</i>         | 4.2*                       | 81                | 288±210                                  | 3.7                        | 118               | 268±202                                  | 3.7-3.8                                      |
| <i>Saduria entomon</i>           | 4.5*                       | 73                | 65±167                                   | 3.9                        | 108               | 55±141                                   | 3.9-4.0                                      |
| <i>Oligochaeta</i> undet.        | 4.5*                       | 67                | 97±138                                   | 4.1                        | 82                | 74±123                                   | 4.1                                          |
| <i>Bylgides sarsi</i>            | 4.9*                       | 28                | 9±18                                     | 4.8                        | 36                | 8±17                                     | 4.0-4.8                                      |
| <i>Halicryptus spinulosus</i>    | 4.9*                       | 52                | 20±26                                    | 4.8                        | 72                | 20±26                                    | 4.5-4.8                                      |

\*-species sensitivity values outside the accuracy range derived from full dataset.

### 4.2.3.3 Species sensitivity values under bottom trawling effects

In total 7 species were reported from the samples taken at the bottom trawling area, within the halocline zone at depth of 60-65 m. The macrozoobenthos structure was notably different in two depths (60-61 m and 63-65 m), therefore the impacted and control samples in these groups were characterized separately (Fig. 10).

Twenty-one sites were classified as impacted and 27 were assigned to the control group according to the nMDS analysis of macrozoobenthos presence/absence data (Fig. 10).

#### 4. Results

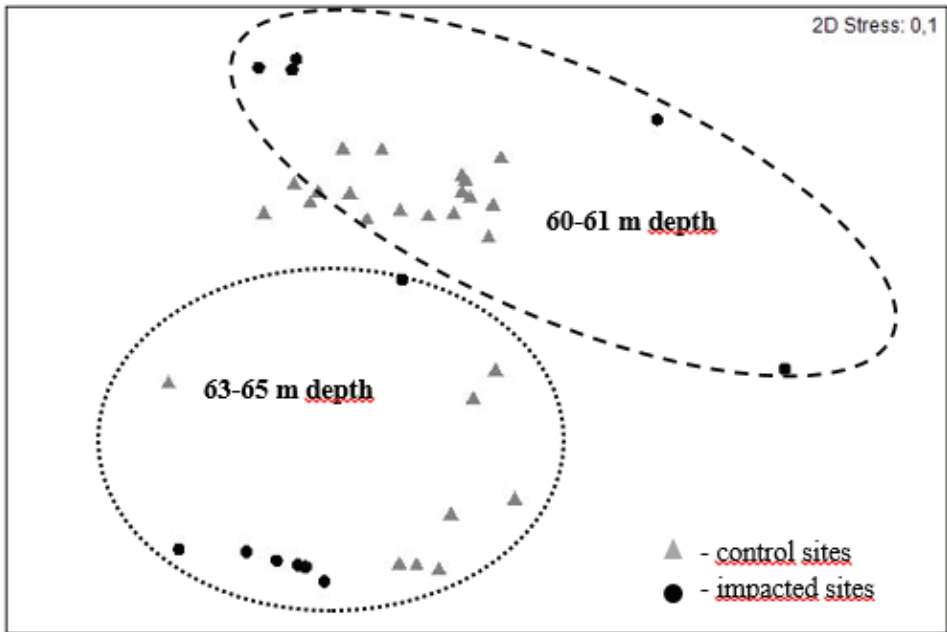


Figure 10. nMDS plot for discrimination of macrozoobenthos sites according to the depth classes and bottom trawling impact.

10 paveikslas. Poveikio ir foninių mėginių rinkimo vietų nMDS analizės klasifikacija pagal makrozoobentosos gausumo duomenis dugninio tralavimo rajone.

In more shallow area at 60-61 m depth, species richness ranged between 4 and 6 taxa at the control sites with the permanent presence of bivalve *L. balthica*, priapulid *H. spinulosus* and polychaete *B. sarsi* as well as occasional occurrence of ostracods (5%). At the impacted sites, 2 to 4 species were recorded with the higher occurrence of *L. balthica* and *H. spinulosus* (27% and 18% respectively). In contrast, at greater depths of 63-65 m only 2-3 taxa (mostly *B. sarsi*, and ostracods) were observed in the control sites, whereas only polychaete *B. sarsi* was found at the impacted sites. Taking into account the entire depth range within the bottom trawling area, the total average of macrozoobenthos abundance was significantly lower (Mann-Whitney test,  $W=186.5$ ,  $p=0.0001$ ) at the impacted sites ( $74 \pm 104$  ind  $m^{-2}$ ) compared to the control sites ( $195 \pm 125$  ind  $m^{-2}$ ).

#### 4. Results

**Table 9.** Comparison of species sensitivity values based on control sites dataset and full dataset (including sites with bottom trawling effects). Accuracy range of the species sensitivity values.

**9 lentelė.** Rūšių jautrumo verčių, skaičiuotų foninių mėginių duomenų rinkiniui ir bendram foninių ir poveikio mėginių duomenų rinkiniui (įtraukiant mėginius su dugninio tralavimo poveikiu), palyginimas. Paskaičiuotas rūšių jautrumo verčių tikslumo intervalas.

| Macrozoobenthos species/taxa  | Control dataset            |                   |                                          | Full dataset               |                   |                                          |                                              |
|-------------------------------|----------------------------|-------------------|------------------------------------------|----------------------------|-------------------|------------------------------------------|----------------------------------------------|
|                               | Species sensitivity values | Number of samples | Species abundance (ind m <sup>-2</sup> ) | Species sensitivity values | Number of samples | Species abundance (ind m <sup>-2</sup> ) | Accuracy range of species sensitivity values |
| <i>Marenzelleria</i> spp.     | 2.0                        | 13                | 6±7                                      | 2.0                        | 15                | 4±7                                      | 2.0                                          |
| <i>Ostracoda</i> undet.       | 2.0                        | 15                | 13±21                                    | 2.0                        | 19                | 15±52                                    | 2.0                                          |
| <i>Halicryptus spinulosus</i> | 3.0*                       | 22                | 22±17                                    | 2.0                        | 26                | 15±18                                    | 1.9-2.0                                      |
| <i>Monoporeia affinis</i>     | 4.9*                       | 8                 | 4±9                                      | 3.5                        | 9                 | 3±7                                      | 3.9                                          |
| <i>Limecola balthica</i>      | 3.0*                       | 19                | 119±102                                  | 2.0                        | 25                | 79±95                                    | 1.9-2.0                                      |
| <i>Bylgides sarsi</i>         | 2.0*                       | 27                | 31±22                                    | 1.0                        | 43                | 26±22                                    | 1.0                                          |

\*-species sensitivity values outside the accuracy range derived from full dataset.

The species sensitivity values were calculated for 6 species using both datasets. The range of sensitivity values was relatively similar in the control and full datasets (2.0-4.9 and 1.0-3.5, respectively) (Table 9), however values calculated from the full dataset were generally lower than those done according to the control sites only. The largest difference of species sensitivity values between the control dataset and accuracy range derived from the full dataset was found for amphipod *M. affinis*, polychaete *B. sarsi*, bivalve *L. balthica* and priapulid *H. spinulosus* (Table 9). In contrast, the sensitivity values of ostracods and spionid *Marenzelleria* spp. did not change between datasets.

### 4.3 Benthic Quality Index assessment

#### 4.3.1 Depth related species sensitivity values effects on Benthic Quality Index

In order to assess the role of depth induced species sensitivity values changes for BQI, values were calculated using species sensitivity values obtained for three different depth intervals (10-30 m, 30-50 m, 50-70 m) and compared with BQI values calculated using species sensitivity values for the entire depth range (10-70 m).

Strong relationships were found between the broad depth range BQI values (10-70 m) and those calculated using the depth specific sensitivity values (10-30 m and 30-50 m) (Fig. 11). The BQI based on the species sensitivity values for 10-70 m depth varied from 3.0 to 5.1 (average  $4.1 \pm 0.5$ ) and were significantly lower (average difference  $-0.2 \pm 0.1$ , Wilcoxon test,  $z = -5.785$ ,  $p < 0.001$ ,  $n = 49$ ) than the BQI estimates based on sensitivities for 10-30 m depth varying in range from 3.2 to 5.1 (average  $4.2 \pm 0.5$ ) (Fig. 11). Although BQI values were underestimated for species sensitivity values from reduced depth range in most cases, the difference between BQI based on the species sensitivity values from two depth ranges decreased considerably from 0.6 to 0.1 towards the higher BQI values. Similarly, broad depth range sensitivity values based BQI were significantly lower (average of difference  $-0.3 \pm 0.1$ , Wilcoxon test,  $z = -6.127$ ,  $p < 0.001$ ,  $n = 50$ ) than those estimated using the 30-50 m depth sensitivity values and varying between 3.3 and 5.7 (average  $4.3 \pm 0.5$ ) (Fig. 11). The difference between the BQI values here, however, increased with the increasing BQI (Fig. 11).

The BQI values based on species sensitivity values for 50-70 m depth varied from 2.3 to 3.5 (average  $2.9 \pm 0.3$ ) and were significantly lower than the BQI estimates based on the species sensitivity values for 10-70 m depth (2.4-5.4) (Fig. 11). In contrast to other two depth intervals, the BQI based on species sensitivities for 50-70 m depth were overestimated up to 2.5 times with no relationship with the BQI values calculated using broad range sensitivity values.



## 4. Results

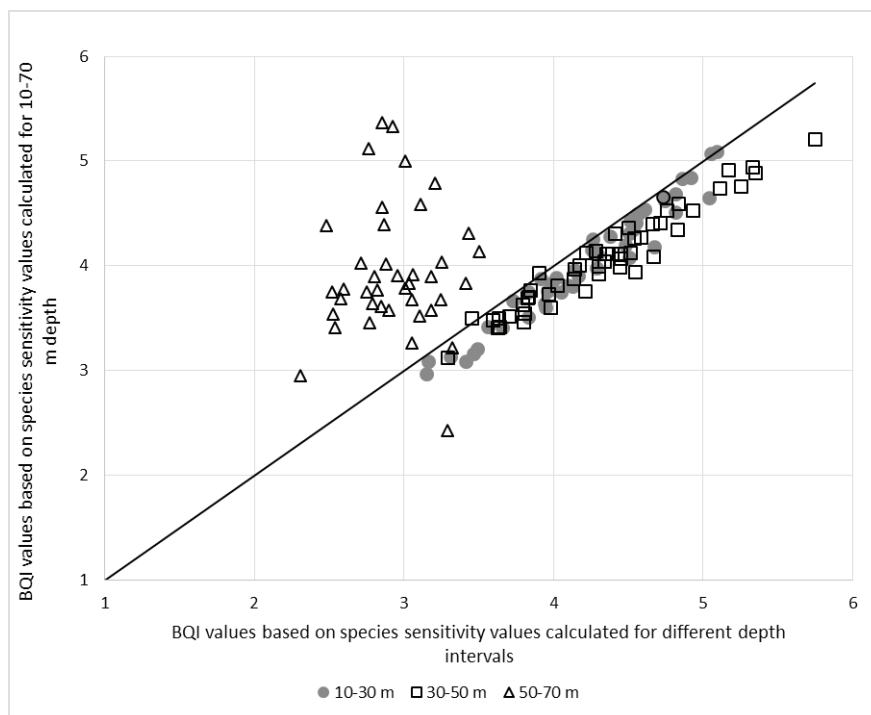


Figure 11. BQI values based on species sensitivity values calculated for 10-30 m, 30-50 m, 50-70 m and 10-70 depth intervals.

11 paveikslas. BQI vertės, apskaičiuotos naudojant rūšių jautrumo vertes iš 10-30 m, 30-50 m, 50-70 m ir 10-70 gylio intervalų.

### 4.3.2 Macrozoobenthos community structure effects on Benthic Quality Index

To test the community structure impact on the benthic quality assessment, the BQI estimates were calculated using different species sensitivity values obtained from: i) samples from community dominated by bivalve *L. balthica*, ii) samples from community dominated by polychaete *Marenzelleria* spp. iii) for pooled data from both communities.

The BQI values based on species sensitivity values obtained for samples from both communities (pooled data) showed a similar range (1.0-3.5) but up to 25% lower values as it was found using sensitivities estimated for *Marenzelleria* spp. community (0.9-2.8) (Fig. 12).

#### 4. Results

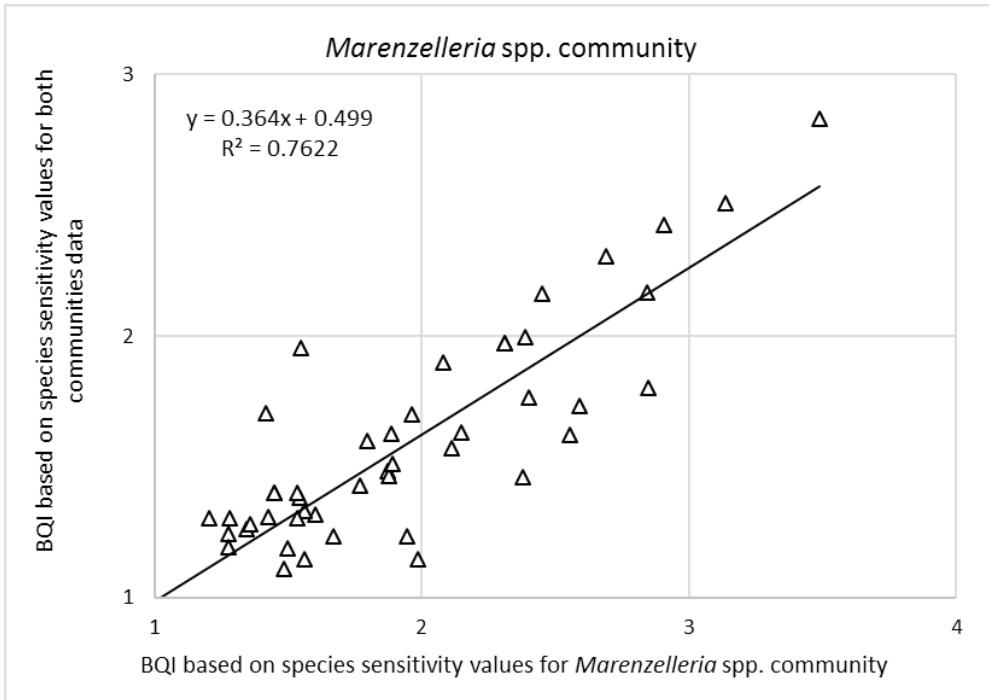


Figure 12. Relationship between BQI values obtained using i) *Marenzelleria* spp. community specific species sensitivity values, and ii) species sensitivity values irrespective of dominant species.

12 paveikslas. Ryšys tarp BQI verčių, apskaičiuotų remiantis i) *Marenzelleria* spp. bendrijos rūšių jautrumo vertėmis ir ii) rūšių jautrumo vertėmis nepriklausomai nuo bendrijos.

The BQI estimates based on species sensitivity values irrespective of the community type (pooled data) varied from 1.6-3.4 (average  $2.5 \pm 0.5$ ) and were significantly lower (Wilcoxon test,  $p < 0.01$ ,  $n = 105$ ) than the BQI values based on species sensitivity values for *L. balthica* community (average  $3.5 \pm 0.4$ ) (Fig. 13). There was weak relationship between these two types of BQI ( $R = 0.296$ ) and the difference between absolute values decreased from 1.5-2 times at their lower range down to 10-15% for the high BQI values.

Overall, the BQI values calculated for species sensitivities obtained from the *Marenzelleria* spp. community dataset (average  $1.5 \pm 0.4$ ) were significantly different (Mann-Whitney test,  $p < 0.01$ ,  $n = 51$ ) and approximately 2 times smaller than the BQI estimates based on the species sensitivity values for *L. balthica* community (average  $3.5 \pm 0.4$ ) (Fig. 12; Fig. 13).

## 4. Results

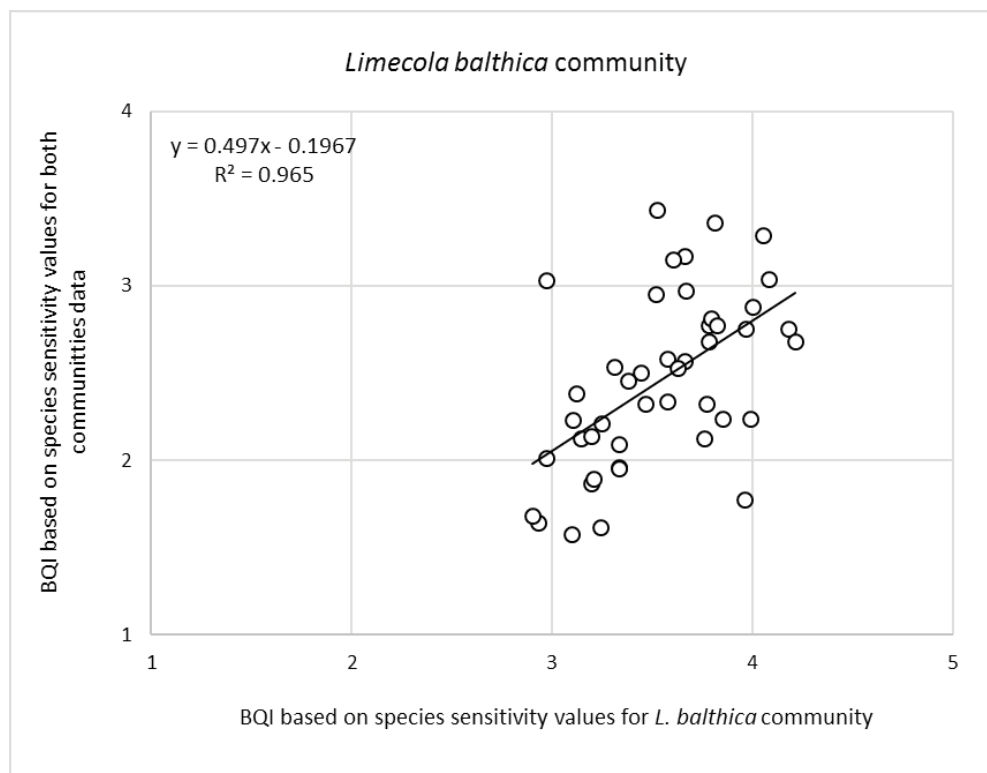


Figure 13. Relationship between BQI values obtained using  
i) *L. balthica* community specific species sensitivity values, and  
ii) species sensitivity values irrespective of dominant species.

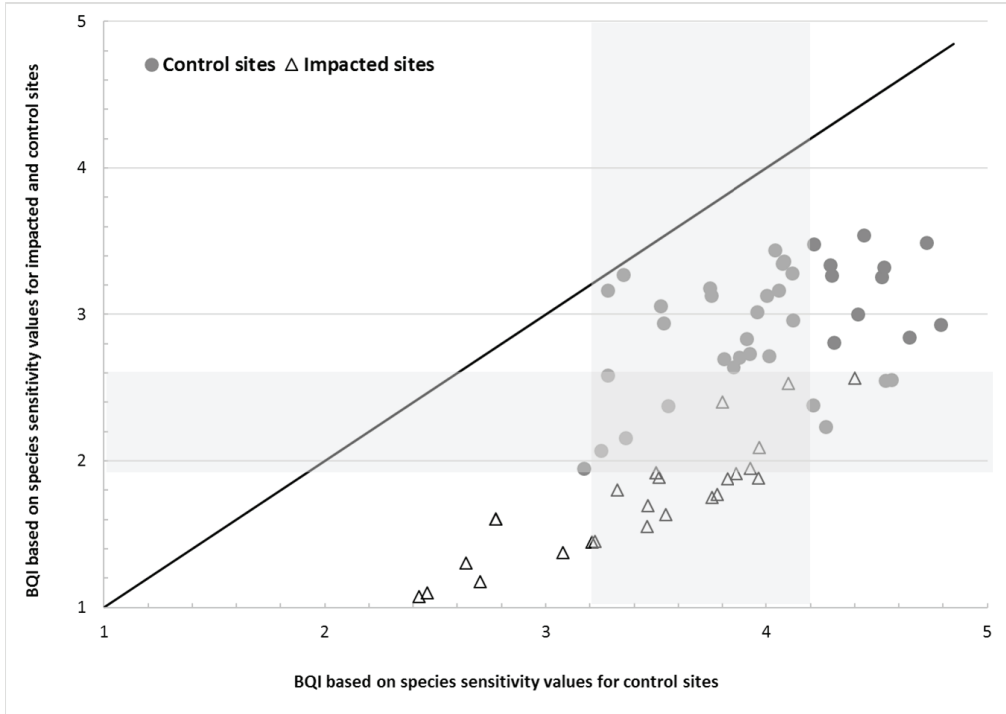
13 paveikslas. Ryšys tarp BQI verčių, apskaičiuotų remiantis  
i) *L. balthica* bendrijos rūšių jautrumo vertėmis ir  
ii) rūšių jautrumo vertėmis nepriklausomai nuo bendrijos

### 4.3.3 Benthic Quality Index response to disturbance

#### 4.3.3.1 Benthic Quality Index values under eutrophication effects

To test the eutrophication effects on sensitivity values and the benthic quality assessment, the BQI values were calculated using the sensitivity values obtained from two different datasets: the control sites only and the full dataset including the control and impacted sites (Fig. 14).

#### 4. Results



*Figure 14.* BQI values calculated using species sensitivity values from control sites only and full dataset (including sites with elevated Chl-*a*) for the eutrophication area. Shaded areas indicate BQI values intervals for overlapping distribution of the control and impacted sites (classified according to nMDS analysis). Line denotes full match of BQI values obtained after using two different sensitivity values.

*14 paveikslas.* BQI vertės apskaičiuotos naudojant jautrumo vertes iš foninių vietų ir bendro (eutrofikacijos poveikio ir foninių) duomenų rinkinių. Pilkas plotas rodo BQI verčių intervalą, kuris sutampa tarp foninių ir poveikio vietų mėginių. Linija rodo BQI verčių, apskaičiuotų naudojant skirtingus rūšių jautrumus, sutapimą.

The BQI values were significantly smaller (Wilcoxon test,  $p < 0.0001$ ,  $z = -9.4$ ,  $n = 65$ ) for species sensitivity values from the full dataset compared to those calculated when using the species sensitivity values from control sites only. At the impacted samples, BQI varied from 2.4 to 4.1 based on the sensitivity values calculated from the dataset of control sites (average  $3.4 \pm 0.5$ ) and from 1.1 to 2.5 (average  $1.7 \pm 0.4$ ) based on the sensitivity values calculated from the full dataset i.e. taking into account the samples with eutrophication effects (Chl-*a*  $> 6-8 \text{ mg/m}^3$ ).

The discrimination of the impacted and control sites is better when the species sensitivity values from the full dataset are used, i.e. the BQI values overlap range is

## 4. Results

narrower (between 1.9 and 2.6) compared to that observed when using the sensitivity values from the control sites only (between 3.1 and 4.1). Consequently, the number of observations within these ranges is much lower in case of the full dataset sensitivity values (28% versus 62% for control sites dataset species sensitivity values).

Below the BQI values threshold of 1.9-3.2 (depending on the dataset used for species sensitivity values calculations) only the impacted sites were observed, however it was only 42% of all impacted samples that fall into this interval. In contrast, the BQI values above 2.5-4.1 exclusively indicated the unimpacted sites and contain 48% of the control samples according to the control and full dataset sensitivity values (Fig. 14).

### 4.3.3.2 Benthic Quality Index values under dredge spoil dumping effects

The BQI values were calculated using the sensitivity values obtained from two different datasets: the control sites only and the full dataset including control sites and impacted sites with dredge spoil dumping effects (Fig. 15). Overall, the BQI values were significantly smaller (Wilcoxon test,  $p < 0.01$ ,  $n = 118$ ) for species sensitivity values from the full dataset compared to those calculated when using the species sensitivity values from the control sites only.

At the impacted sites, the BQI values varied from 1.9 to 3.8 (average  $3.0 \pm 0.5$ ) based on the sensitivity values calculated from the control sites and from 1.7 to 3.6 (average  $2.8 \pm 0.2$ ) based on the sensitivity values calculated from the full dataset taking into account sites with dredge spoil dumping effects. In contrast, the BQI values at the control sites varied from 2.8 to 3.9 and from 2.7 to 3.7 when using species sensitivity values estimated from control and full datasets respectively. Below the BQI values threshold of 2.6-2.7 (depending on dataset used for sensitivity values calculations) only the impacted sites were observed, however it was only 27% of all impacted samples that fall into this interval. In contrast, the BQI values above 3.6-3.7 exclusively indicated the unimpacted sites, while the BQI values range between 2.6 and 3.6 contained 90% of samples with 43% and 80% of those classified correspondingly as impacted and control sites respectively (Fig. 15).

#### 4. Results

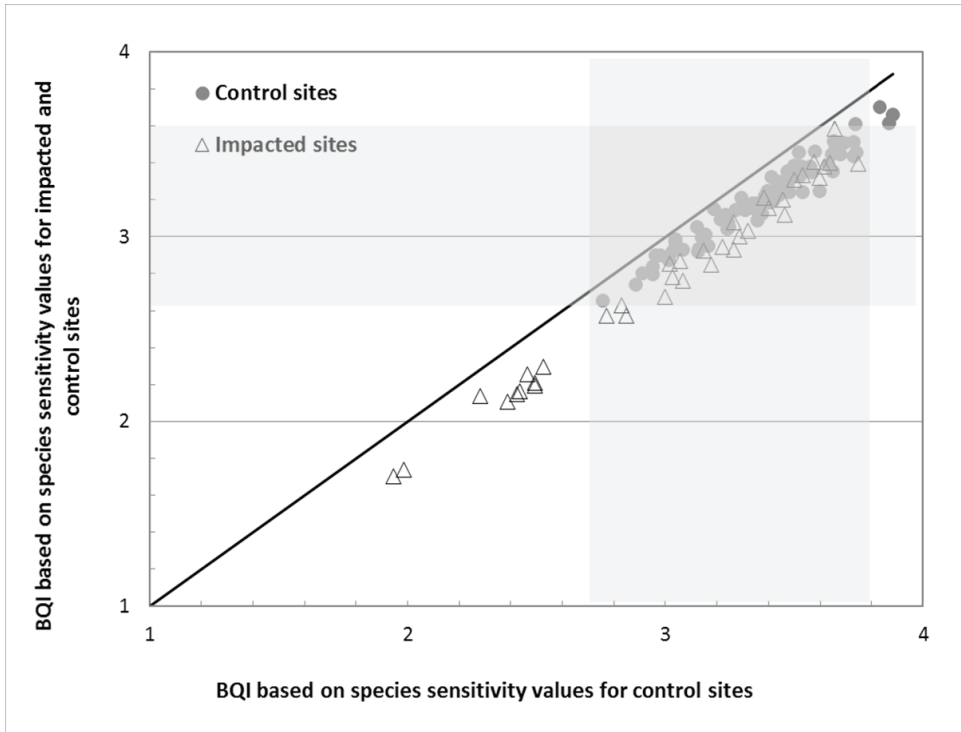


Figure 15. BQI values calculated using species sensitivity values from control sites only and full dataset (including sites impacted by dredge spoil dumping effect) for the dredge spoil dumping area. Shaded areas indicate BQI values intervals for overlapping distribution of control and impacted sites (classified according to nMDS analysis). Line denotes full match of BQI values obtained after using two different sensitivity values.

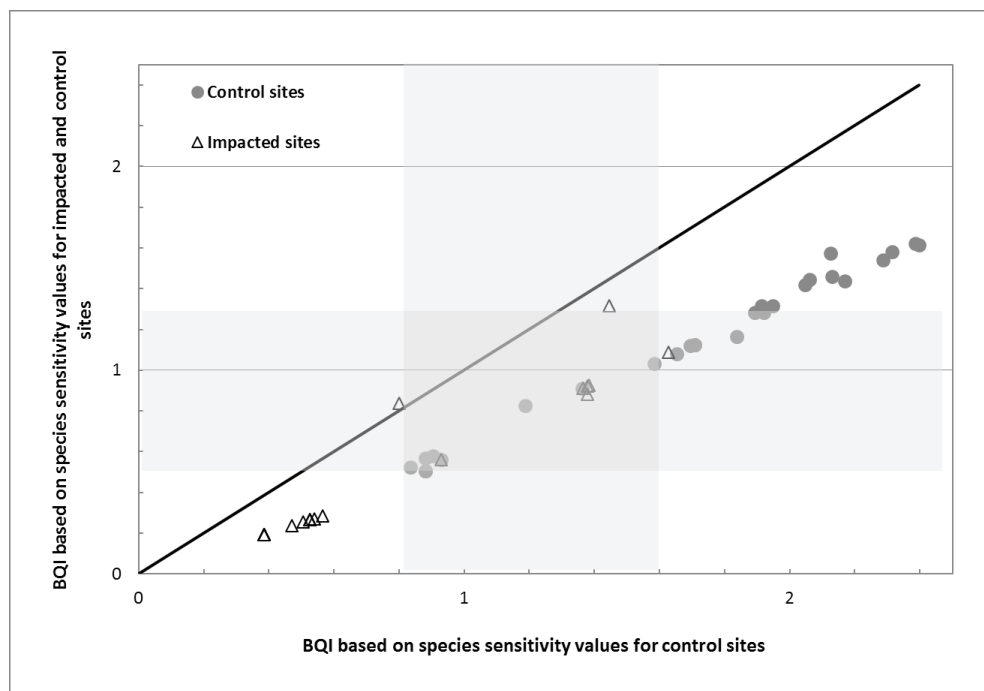
15 paveikslas. BQI vertės apskaičiuotos naudojant jautrumo vertes iš foninių vietų ir poveikio ir foninių duomenų rinkinių, surinktų grunto pylimo rajone. Pilkas plotas rodo BQI verčių intervalą, kuris sutampa tarp foninių ir poveikio mėginių. Linija rodo BQI verčių, apskaičiuotų naudojant skirtingus rūšių jautrumus, sutapimą

##### 4.3.3.3 Benthic Quality Index values under bottom trawling effects

The BQI and species sensitivity values under bottom trawling effects were analysed using two different datasets: the control sites only and the full dataset including the control sites and the impacted sites (Fig. 16). The BQI values based on the species sensitivity values from the control sites ranged from 0.4 to 2.4 (average  $1.3 \pm 0.7$ ) and resulted in significantly lower values (Wilcoxon test,  $p < 0.001$ ,  $n = 48$ ) compared to the BQI values range of 0.2 to 1.6 (average  $0.9 \pm 0.5$ ) estimated for the species sensitivity values based on the full dataset.

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Similarly, analyzing the group of impacted sites separately, the BQI values were significantly smaller by 33-50% (Wilcoxon test,  $p < 0.001$ ,  $n = 21$ ) for the species sensitivity values from full dataset compared to those based on the control dataset. The difference between the BQI values estimated from the full and control dataset was increasing towards higher BQI values indicating higher risk of getting overestimated assessment values if the disturbance gradient is not covered by sampling and species sensitivity values are exclusively estimated from undisturbed samples.



*Figure 16.* BQI values calculated using species sensitivity values from control sites and full datasets (including sites with bottom trawling effects) for bottom trawling area. Shaded areas indicate BQI values intervals for overlapping distribution of control and impacted sites (classified according to nMDS analysis). Line denotes full match of BQI values obtained after using two different sensitivity values.

*16 paveikslas.* BQI vertės apskaičiuotos naudojant jautrumo vertes iš foninių ir poveikio ir foninių duomenų rinkinių, surinktų dugninio tralavimo rajone. Pilkas plotas rodo BQI reikšmių intervalą, kuris sutampa tarp foninių ir poveikio vietų. Linija rodo BQI verčių, apskaičiuotų naudojant skirtingus rūšių jautrumus, sutapimą.

## 4. Results

Depending on the dataset used for species sensitivity values calculation, the BQI below 0.5-0.8 exclusively were found for the impacted sites (29% of sites, excluding defaunated sites) and the BQI values above 1.3-1.6 indicated the control sites (19-35% of sites) (Fig. 16) following nMDS classification results. The BQI values range covering values estimated from the control and impacted sites was similar using both datasets; however, 36% of sites fall into this interval using the control dataset versus 52% of sites assessed with the full dataset.

### 4.4 Validation of the Benthic Quality Index

#### 4.4.1 Water quality class boundaries according to the species sensitivity groups

Sensitivity values were assessed for 11 coastal macrozoobenthos species for depths down to 20 m. The resulting sensitivity values ranged from 2.3 to 5.8 and species were grouped into 4 groups from very tolerant to very sensitive using the arbitrary boundaries of whole numbers to distinguish between groups (Table 10). The relative abundance trends of these groups along the axis of the ranked BQI values (1.1-3.9) have been used to describe water quality classes and their boundaries (Fig. 17; Table 11).

**Table 10.** Classification of macrozoobenthos species sensitivity values and composition of defined sensitivity groups for the coastal waters.

**10 lentelė.** Lietuvos Baltijos jūros priekrantės makrozoobentoso rūšių jautrumo verčių klasifikacija ir jautrumo grupių sudėtis.

| Macrozoobenthos species/taxon | Species sensitivity values in Lithuania coastal waters | Species sensitivity groups description   |
|-------------------------------|--------------------------------------------------------|------------------------------------------|
| <i>Marenzelleria</i> spp.     | 2.3                                                    | Very tolerant<br>(Sensitivity group I)   |
| <i>Corophium volutator</i>    | 2.3                                                    |                                          |
| <i>Pygospio elegans</i>       | 2.5                                                    |                                          |
| <i>Limecola balthica</i>      | 3.1                                                    | Tolerant<br>(Sensitivity group II)       |
| <i>Hediste diversicolor</i>   | 3.8                                                    |                                          |
| <i>Mya arenaria</i>           | 4.4                                                    | Sensitive<br>(Sensitivity group III)     |
| <i>Oligochaeta</i> undet.     | 4.4                                                    |                                          |
| <i>Hydrobia</i> sp.           | 4.4                                                    |                                          |
| <i>Cerastoderma glaucum</i>   | 4.8                                                    |                                          |
| <i>Bathyporeia pilosa</i>     | 5.3                                                    | Very sensitive<br>(Sensitivity group IV) |



#### 4. Results

**Table 11.** Characterisation of water quality classes according to relative abundance of four macrozoobenthos sensitivity groups and BQI values.

**11 lentelė.** Vandens kokybės klasių aprašymas pagal BQI vertes ir makrozoobentosos jautrumo grupių santykinio gausumo pokyčius.

| Water quality class | Water quality class description                                                                                                                                                                                                     | Water quality class threshold according to BQI |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| Bad                 | Relative abundance of very tolerant species was permanently higher than 90%, while sensitive species do not exceed 6% of the total macrozoobenthos abundance. Relative abundance of very sensitive species was always less than 1%. | < 1.5                                          |
| Poor                | Relative abundance of very tolerant species was between 80 and 90%, sensitive species could reach up to 15% of the total abundance. Abundance of very sensitive species was always less than 5%.                                    | 1.5 - 2.1                                      |
| Moderate            | Relative abundance of very tolerant species was between 50 and 80%, whereas sensitive species could reach 6-50% of the total abundance.                                                                                             | 2.1 - 2.7                                      |
| Good                | Very tolerant macrozoobenthos species usually make less than half of all benthic organisms. Occurrence of very sensitive species was higher than 50%.                                                                               | 2.7 - 3.3                                      |
| High                | Relative abundance of tolerant species usually exceeds 20%, however very sensitive species were permanently present.                                                                                                                | > 3.3                                          |

The relative abundance of very tolerant macrozoobenthos taxa (spionids *Marenzelleria* spp., *P. elegans* and crustacean *C. volutator*) was typically above 94% for the samples with the BQI values below 1.3 (Fig. 17, Table 11). The relative abundance of this group was consistently decreasing at the increasing BQI values and typically was below 50% for BQI values higher than 2.8.

The group of tolerant macrozoobenthos species consisting of bivalve *L. balthica* and polychaete *H. diversicolor* had the highest relative abundance at the BQI values range from 2.0 to 3.3 (Fig. 17, Table 11); however, the relative abundance of this group only rarely exceeded 30% in the studied samples. The relative abundance of tolerant species was below 10% at the BQI values higher than 3.5.

The relative abundance of sensitive polychaete species (gastropod *Hydrobia* sp., bivalves *C. glaucum*, *M. arenaria* and oligochaetes) increased with the increasing BQI values (Fig. 17, Table 11). The BQI values were below 2.0 at the relative abun-

#### 4. Results

dance of these species lower than 10%. However, the BQI values were always higher than 3.0 when the relative abundance exceeded 20%.

*B. pilosa* and rarely present polychaete *S. shrubsolii* belong to the very sensitive species group (Fig. 17, Table 11), which average relative abundance was very low (~1%) and usually less than 9%. There was a weak trend in the relative abundance of this species group under the increasing BQI values, the highest values (>10%) were typically reached at the BQI values exceeding 2.9.

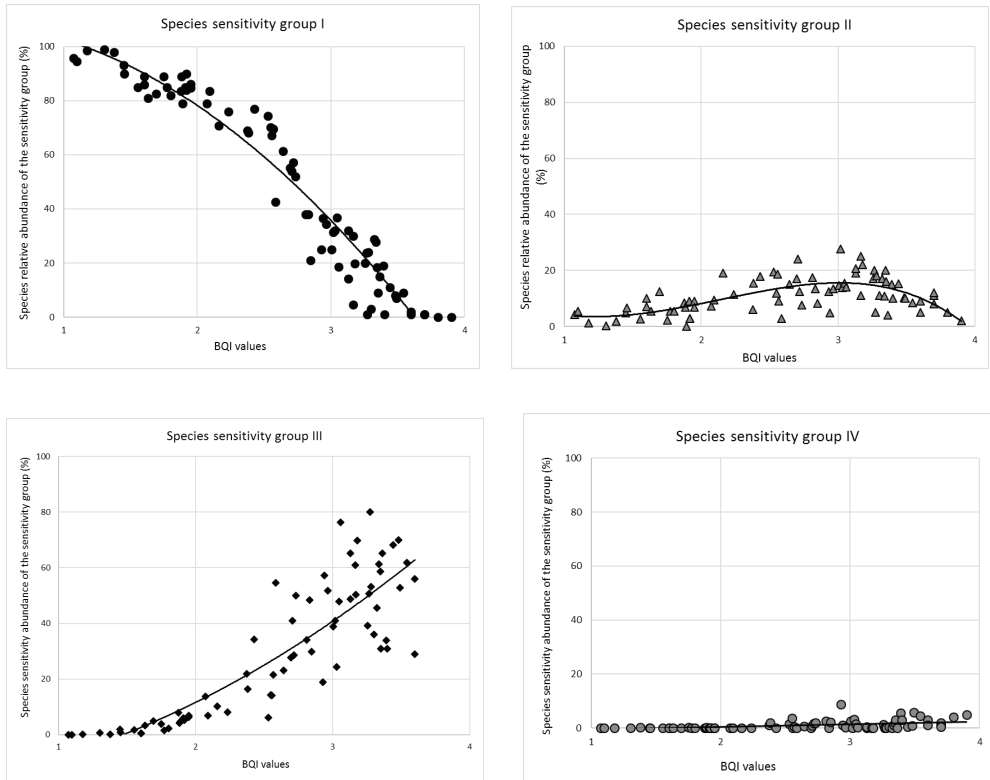


Figure 17. Relative abundance (%) of macrozoobenthos sensitivity groups (I-IV) and corresponding BQI values for the coastal waters.

17 paveikslas. Makrozoobentos rūšių jautrumo grupių (I-IV) santykinio gausumo ryšys su BQI reikšmėmis priekrantėje.

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### 4.4.2 Water quality class boundaries according to the regression analysis

The validation of BQI values was carried out defining its relationship with Chl-*a* for the coastal waters and plume area (Fig. 18). In the plume area Chl-*a* was 6-7 times higher than in the coastal sites ( $27.4 \pm 2.0$  and  $4.4 \pm 1.0$  mg/m<sup>3</sup> respectively), however, its variability in both environments was comparable (coefficient of variation 0.05 and 0.02 mg/m<sup>3</sup> respectively). The BQI values were also similar in the plume area (average  $2.2 \pm 0.4$ ) and coastal waters (average  $2.8 \pm 0.4$ ) (Fig. 18).

A strong and significant relationship ( $R=0.861$ ,  $p=0.0001$ ) was found between Chl-*a* and BQI values for the coastal waters sites, where the increase in Chl-*a* by 1 mg/m<sup>3</sup> resulted in average decrease of BQI values by 0.6 units. A considerably weaker and not significant relationship between BQI values and Chl-*a* was obtained for the plume area ( $R=0.396$ ,  $p=0.1450$ ) with the relatively high BQI values variability at similar Chl-*a* values (Fig. 18).

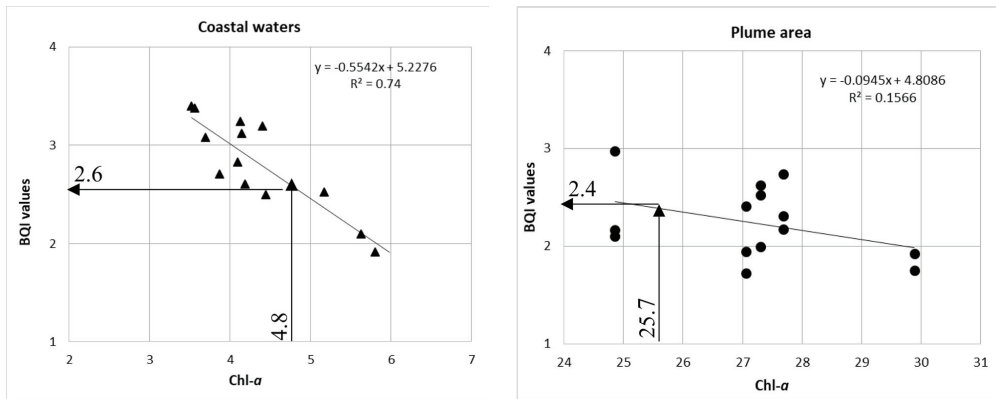


Figure 18. Relationship between BQI values and Chl-*a* for the coastal waters (left) and plume area (right).

18 paveikslas. Ryšys tarp BQI ir Chl-*a* koncentracijos priekrantės (kairėje) ir Kuršių marių vandenų sklaidos zonoje jūroje (dešinėje).

Both regressions (Fig. 18) were used for the validation of BQI values threshold between moderate and good water quality classes for the coastal waters and the plume area. For this purpose the BQI threshold estimates for both areas were back-calculated using Chl-*a* values, which were independently set for good-moderate quality class threshold combining expert judgment and statistical exploitation of the monitoring data ( $4.8$  mg/m<sup>3</sup> for the coastal waters and  $25.7$  mg/m<sup>3</sup> for the plume area, see Langas et al., 2009 for details). The back-calculated BQI values were highly consistent with

#### 4. Results

those, which have been derived according to the relative abundance of different sensitivity groups (Table 12).

**Table 12. Comparison of back-calculated and determined thresholds of BQI values with Chl-*a* for the good-moderate water quality class boundaries for the coastal waters and plume area.**

**12 lentelė. Geros-vidutinės vandens būklės slenkstinių reikšmių apskaičiavimas pagal BQI verčių ir Chl-*a* koncentracijos santykį bei nustatymas pagal BQI reikšmių ir makrozoobentosos rūšių jautrumo grupių santykį priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje.**

| Water quality class threshold | Coastal waters                    |                           |                           |                          | Plume area                       |                           |                           |                          |
|-------------------------------|-----------------------------------|---------------------------|---------------------------|--------------------------|----------------------------------|---------------------------|---------------------------|--------------------------|
|                               | Chl- <i>a</i> mg /m <sup>-3</sup> | BQI <sub>bc-calc.</sub> * | BQI <sub>determ.</sub> ** | BQI <sub>diff.</sub> *** | Chl- <i>a</i> mg/m <sup>-3</sup> | BQI <sub>bc-calc.</sub> * | BQI <sub>determ.</sub> ** | BQI <sub>diff.</sub> *** |
| good - moderate               | 4.8                               | 2.6                       | 2.7                       | 0.1                      | 25.7                             | 2.4                       | 2.7                       | 0.3                      |

\*-BQI threshold back-calculated from Chl-*a* threshold (Langas et al., 2009) according to the relationship between BQI and Chl-*a* (summer average) for the coastal waters and plume area (Fig. 18).

\*\* -BQI threshold determined according to dynamics of relative abundance of macrozoobenthos sensitivity groups along the BQI gradient (Fig. 17).

\*\*\*-difference between back-calculated and determined thresholds of BQI values.

The BQI validation with TN and TP summer concentration was carried out applying linear regression. No significant relationships were detected between the BQI values and TN or TP either for the plume area or coastal waters (Fig. 19; 20). The calculated BQI values ranged from 1.7 to 3.4 with no apparent difference between the plume area and the rest of the coastal waters. The average TN concentration was significantly higher in the plume area than in the coastal waters ( $51 \pm 17$  µg/l and  $35 \pm 11$  µg/l,  $t = -3.783$ ,  $p = 0.0006$ ) (Fig. 19). The importance of the Curonian lagoon outflow for TN is supported by a significant negative relationship between salinity and TN in the plume area ( $r = -0.72$ ,  $p < 0.001$ , salinity range between 3.3 and 7.1), while at the coastal waters this relationship was negligible ( $r = -0.02$ ,  $p < 0.001$ , salinity range between 6.3 and 7.4). TP was similar in the plume area and coastal waters with no significant differences between average values ( $3.8 \pm 1.0$  µg/l and  $3.4 \pm 1.0$  µg/l respectively) and a very weak negative relationship with salinity.

## 4. Results

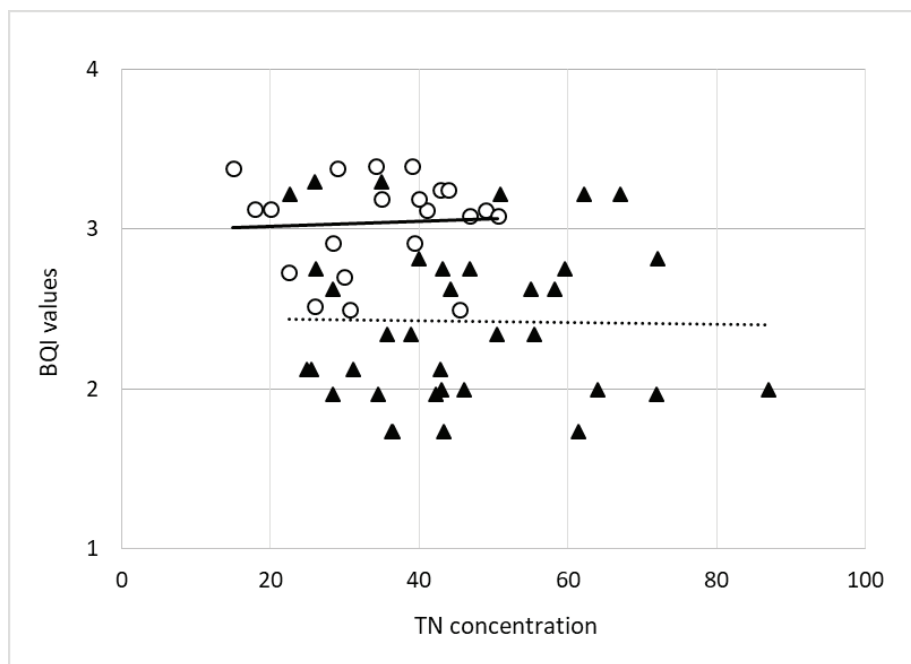


Figure 19. BQI values versus TN with fitted linear model trend lines for the sites at the coastal waters (○) and in the plume area (▲).

19 paveikslas. Ryšys tarp BQI reikšmių ir bendro azoto koncentracijos priekrantėje (○) ir Kuršių marių vandenų sklaidos zonoje jūroje (▲).

### 4.4.3 Water quality class boundaries according to the Signal detection theory

The analysis based on the Signal detection theory was performed separately for the coastal waters and the plume area. An acceptable BQI values response ( $AUC \geq 0.70$ , sensu Hale and Heltshe (2008)) was revealed to all three analysed eutrophication parameters measured at the coastal waters. The best BQI values response was found for Chl-*a* ( $AUC=0.75$ ) and TP ( $AUC=0.74$ ) (Fig. 21). Within the plume area, however the index response to Chl-*a* and TP was qualified as poor ( $AUC=0.56$  for both parameters), but excellent for TN ( $AUC=0.87$ ) (Fig. 21).

The most accurate BQI threshold, according to the sum of index sensitivity and specificity values estimated from the index response to Chl-*a* (i.e. ROC curves), was 2.56 (index specificity and sensitivity 0.75 and 0.86 respectively; Fig. 22). In this 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 preva-

#### 4. Results

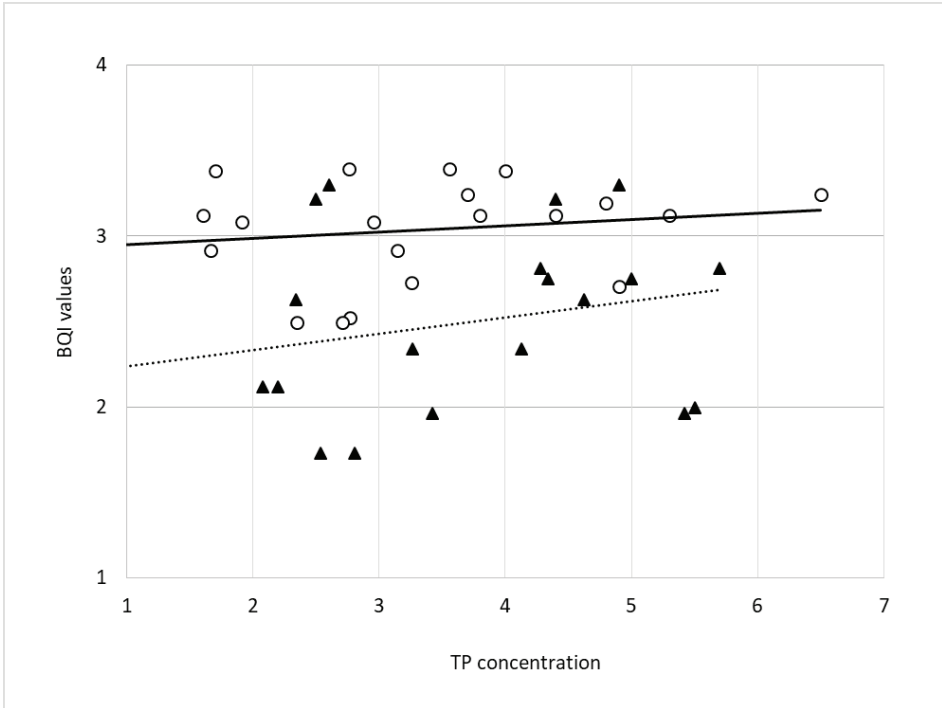


Figure 20. BQI values versus TP with fitted linear model trend lines for the sites taken at the coastal waters (○) and in the plume area (▲).

20 paveikslas. Ryšys tarp BQI reikšmių ir bendros fosforo koncentracijos priekrantėje (○) ir Kuršių marių vandenų sklaidos zonoje jūroje (▲).

lence, 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. 22; Table 13).

NPV values better explain index threshold variation at a healthy environment, while PPV values in disturbed areas. For instance, when applying a strict BQI threshold at 2.45 for threshold between the good and moderate quality 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 resulted in a comparatively low PPV and high NPV (80% and 100% respectively) (Table 13).

## 4. Results

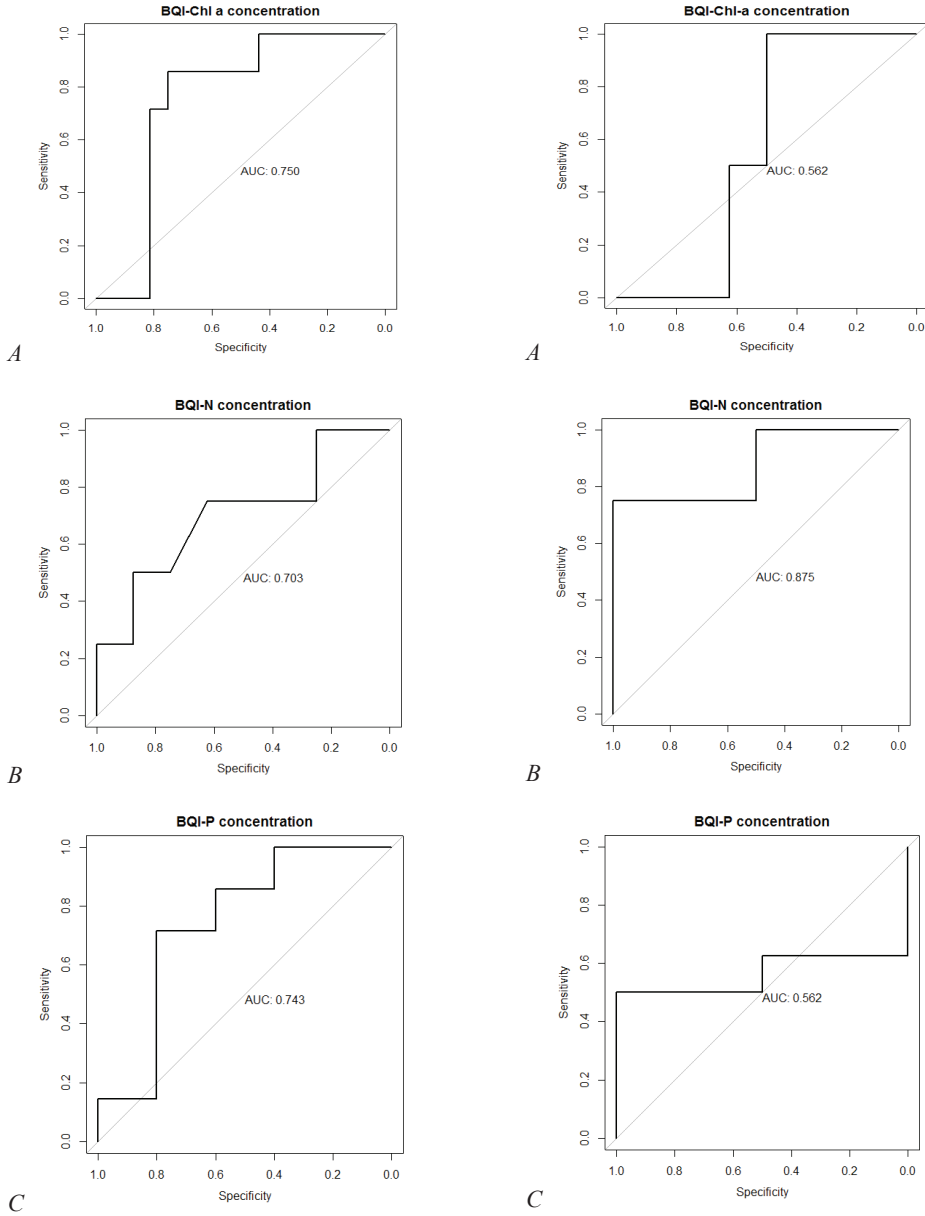


Figure 21. ROC curves for BQI values response to Chl-a (A), TN (B) and TP (C) in the coastal waters (left column) and the plume area (right column).

21 paveikslas. ROC kreivės apibūdinančios BQI atsaką į Chl-a, bendro azoto ir bendro fosforo koncentracijas priekrantėje (kairys stulpelis) ir Kuršių marių vandenų sklaidos zonoje jūroje (dešinys stulpelis).

#### 4. Results

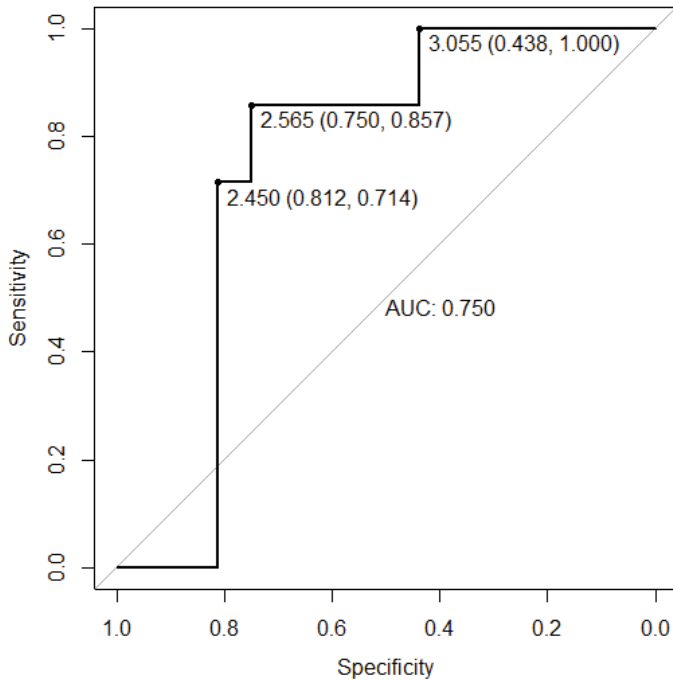


Figure 22. ROC curves for the BQI response to the Chl-*a* at the coastal waters. The steps denote the proposed threshold values (strict-2.45, the most accurate-2.56 and lenient-3.05). Numbers in brackets indicate index specificity and sensitivity values respectively.

22 paveikslas. BQI atsako į Chl-*a* poveikį ROC kreivės priekrantės zonai. Vandens kokybės klasių slenkstinės vertės (griežta slenkstinė vertė-2,45; labiausiai tiksli vertė-2,56 ir „lanksti“ vertė-3,05). Skaičiai skliausteliuose rodo indekso jautrumo ir specifiškumo reikšmes.



#### 4. Results

**Table 13.** BQI thresholds for the coastal waters based on the response to Chl-*a*, with the corresponding estimates of prevalence, index specificity, index sensitivity, PPV and NPV (based on SDT approach).

**13 lentelė.** BQI slenkstinės reikšmės priekrantės zonai pagal atsaką į Chl-*a* koncentracijos poveikį ir jas atitinkančios paplitimo, indekso jautrumo, specifiškumo, teigiamos nuspėjamos reikšmės, neigiamos nuspėjamos reikšmės (apskaičiuotos pagal Signalo aptikimo teoriją).

| BQI values thresholds  | Prevalence of the target Chl- <i>a</i> values (between good and moderate conditions) | Index specificity | Index 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     |



# 5

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## Discussion

The BQI is one of the most widely used multimetric indices for macrozoobenthos status assessment (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 as long as the assigned species' 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 various environmental conditions (Schiele et al., 2016; Leonardsson et al., 2015). Its performance was shown to be affected by the salinity and depth gradients (Labruno et al., 2006; Zettler et al., 2007; Schiele et al., 2016) and the presence of invasive species (Zaiko and Daunys, 2015), however, the BQI responsiveness to different anthropogenic pressures as well as importance of responding community type was not tested. When testing an indicator's responsiveness, ideally the assessment should be performed along the gradient of the selected pressure, excluding any untargeted disturbances (noise effects). This is, however, an unlikely case when working with typical field data and particularly with those from the coastal areas, where multiple natural and anthropogenic disturbances are often simultaneously present. In spite of this, the current study is aimed at tracing BQI values changes by exploring the variability of species sensitivity values in the context of different environmental conditions, benthic communities and anthropogenic disturbances.

## 5.1 Changes of species sensitivity and Benthic Quality Index values along the environmental gradients

Accurate species sensitivity estimates have been declared as being one of the crucial elements in the status assessment of marine waters (Mearns and Word, 1982; Borja et al., 2000; Muxika et al., 2007; Bellan, 2008; HELCOM, 2013). However, despite the acknowledged scientific robustness of the approach to the base sensitivity assessment on mathematical formula (Leonardsson et al., 2015), considerable differences in species sensitivity values were found for different Baltic Sea sub-regions, salinity and depth classes (Zettler et al., 2007, Šiaulyš et al., 2011; Villnäs et al., 2015; Leonardsson et al., 2016; Schiele et al., 2016).

At the Baltic Sea scale, the salinity gradient is the predominant factor controlling the distribution patterns of benthic organisms. The number of macrozoobenthos species declines from the south-west to the north and consequently affects structure and diversity of benthic communities. This phenomena has been demonstrated as having significant effects on BQI and species sensitivity values (Zettler et al., 2007; Schiele et al., 2016). At the regional (sub-basin) scale, however, depth becomes also important for larger shifts in benthic communities. So far depth related changes in sensitivity values were resolved by arbitrarily dividing datasets following the depth zonation into coastal, intermediate and halocline areas (Schiele et al., 2016).

The results of this study showed the relatively consistent sensitivity values for only 4 of 16 species along the analysed depth gradient. Sensitivity values were the most stable for the species with the highest abundance and occurrence: spionids *Marenzelleria* spp., *P. elegans* and bivalve *L. balthica*. The results also demonstrated the decreasing species sensitivity values towards deeper parts of the study area and particularly at the depth of 50 m when approaching the upper boundary of the halocline. Similar results were found for Swedish waters for the species sensitivity values and BQI variation at the 60-100 m depth range (Leonardsson et al., 2016). Additionally, the critical depth of 30-40 m identified for both groups of shallow and deeper living species indicate potentially overestimated species sensitivity values primarily for coastal species. This was demonstrated for bivalve *C. glaucum* sensitivity values, when its sensitivity values increased with increasing depth. Similarly, sensitivity values of deeper water species (e.g. isopod *S. entomon*) also increased outside their typical depth distribution range towards shallow areas. In general, the results on decreasing species sensitivity values towards deeper areas coincide with the phenomena of lower sensitivity values and decreasing ratio between high sensitivity values and low sensitivity values species with decreasing salinity (Schiele et al., 2016). It seems that both dominance of tolerant species due to the increased stress towards greater depths as well as generally lower abundance and species richness due to lower environmental heterogeneity in deeper areas contribute to numerically low sensitivity and BQI values. Due to this,

## 5. Discussion

water quality status assesment should obviously be carried out for coastal waters and halocline zone individually.

Depth and salinity are important factors capable to shape benthic communities, but other important attributes of the seabed environment, such as food supply, oxygen concentrations, currents, temperature, turbidity, and substrate composition may also influence macrozoobenthos distribution in the Baltic Sea (e.g. Bromley, 1996; Olenin, 1997; Pearson and Rosenberg, 1978; Bonsdorff, 2006). These, in turn, may also induce high variability of the species sensitivity values. For example amphipod *M. affinis* was defined as very sensitive for the Swedish east coast and very tolerant in the central Baltic (Schiele et al., 2016), but again it is being ranked among the most sensitive “deep” living species based on the results of this study. It typically occurs in the depths of 30–120 m., but its dominance in the central Baltic is restricted to the depth range of 40–60 m (Olenin, 1997; Daunys et al., 2015). In case of the upper distribution boundary of *M. affinis*, the species occurrence will be associated with diverse macrozoobenthos communities and therefore its sensitivity values will obviously attain higher values. In contrast, at its lower depth range boundary *M. affinis* will occur in relatively low abundance and poor diversity communities, which will support lower species sensitivity value. In addition, distribution limiting factors may correlate with other depth related disturbances and their effects can be hardly distinguishable. For instance, low *M. affinis* abundance within the halocline and below agrees well with its negative response to oxygen demand found after experimental manipulations (e.g. Johansson, 1997). At the same time, markedly lower level of genetic diversity in the offshore *M. affinis* population compared to the coastal one was also reported (Guban et al., 2016) indicating potentially different species ability to survive or respond to disturbances. Literature data also suggest multiple interactions of *M. affinis* with local community species. Intraspecific competition between adults and juveniles can be responsible for the regulation of the population, and the species is susceptible to predation by relatively shallow *B. sarsi* and priapulid *H. spinulosus* (Abrams et al., 1990, Hill, 1992). An the inverse relationship between *L. balthica* and *M. affinis* due to ingestion of the newly settled bivalve spat together with the bottom sediment was also observed in the field and tested in the laboratory experiments (Segerstråle, 1962; Ejdung and Elmgren, 1998; Rousi et al., 2013). It was also shown (Zaiko and Daunys, 2015) that the presence and impact of a powerful habitat forming species may significantly alter the benthic structure and BQI characteristics. The ability of habitat modifying species to form local patches of biological diversity may bias the results of benthic quality assessment by inducing higher BQI values, but specific alghorithms are possible to apply in order to disentangle and compensate for these effects.

In this study, significant differences in species sensitivity values were found when analyzing two main coastal waters communities dominated by bivalve *L. balthica* and polychaete *Marenzelleria* spp. Generally, species diversity and abundance in *Marenzelleria* spp. community was lower compared to *L. balthica* community and this re-

sulted in almost 50% lower sensitivity values for the same species being recorded in polychaete dominated areas. These differences can be explained by substrate differences, however these have not been precisely studied using grain-size analysis. The BQI values also showed significant differences between those obtained from two different communities and values calculated for the pooled data from both communities together. This obviously reflects species sensitivity shifts between the analysed datasets and may result in a significantly different water quality status assessment. Therefore, during the water quality assessment it is necessary to analyse the patterns of macrozoobenthos abundance changes and consider the potential response of BQI to the natural variability of community structure and species richness in the area.

Our analysis of the sensitivity changes and sub-sequent BQI response to the depth gradient and environmental heterogeneity within the same depth range demonstrate, that causal relationships between the anthropogenic impact and species status might not be straightforward, therefore all aspects mentioned above should be considered before taking decisions on contradicting sensitivity values.

### **5.2 Changes of species sensitivity and Benthic Quality Index values according to the coverage of disturbance gradient by samples**

The BQI formula is based not only on the species tolerance to a disturbance but also on its capability to coexist with other species. A high sensitivity value means that the species occurs in a high diversity community and has a high competitive ability; it is seldom found in species poor and disturbed environments. A low sensitivity value on the other hand means that the species has been found predominantly in species-poor environments. This implies that deriving sensitivity values based on samples from rather pristine environments alone is likely to produce higher sensitivity values than if the samples come from disturbed environments. However, if all samples come from a disturbed environment, sensitive species will typically be missing and their sensitivity values can not be calculated.

Species sensitivity values may be highly dependent on the diversity range, covered by the dataset. Absence or insufficient sampling effort in disturbed sites will inadequately reflect the dynamics of a species abundance along the disturbance gradient, therefore it may affect the discrimination of sensitivity groups and bias the overall water quality assessment. It was suggested using at least 20 samples from each of the undisturbed and impacted environments (Leonardsson et al., 2015) in order to properly reflect a disturbance gradient when analyzing the sensitivity and BQI values. In this study the number of samples from the undisturbed and disturbed sample sites varied depending on the analysed pressure, but always fulfilled this condition (27-81 samples from the undisturbed and 21-37 samples from the disturbed sites), with a ratio of the disturbed to undisturbed sites varying between 1:1.3 and 1:2.2. An under-represented disturbance gradient with low diversity macrozoobenthos

## 5. Discussion

communities was simulated by employing control samples with no signs of disturbance in the macrozoobenthos structure against the full dataset containing information from control and impacted sites. The results demonstrated that by including the data from the impacted sites, the estimated species sensitivity values decreased in 40-60% of cases compared to the calculations based on the data from unimpacted sites only. Such a decrease was independent of the number of samples and fell beyond the accuracy range of the sensitivity values generated through the Jackknife resampling. Similarly, the independent study of Leonardson and co-authors (2015) also showed the decrease of sensitivity values with the increasing proportion of samples from disturbed areas.

The estimated sensitivity values were affected by the dataset change independently of the species abundance. For instance, by replacing the control dataset with the full dataset containing samples from the impacted sites (i.e. extending ES50 axis towards lower range) the sensitivity values of low abundance (e.g. *M. affinis*) and high abundance (e.g. *P. elegans*) species were changed to a similar extent. However, the sensitivity values of typically resistant species such as *L. balthica* were more affected by the dataset change compared to the consistent sensitivity values of spionid *P. elegans*, known as having highly negative reaction to the dredge spoil dumping in the region (Olenin, 1992). Other resistant species to the dredge spoil dumping effects such as isopod *S. entomon* also demonstrated the largest shift in sensitivity values towards lower value after including samples from the disturbed sites into the analysis. Under eutrophication effects for the species like *C. glaucum* the sensitivity values did not change between the analysed datasets showing the sensitive species response to the eutrophication process (in terms of Chl-*a* concentration increase), while the *L. balthica* sensitivity values were affected by the dataset change as well as under the dredge spoil dumping or bottom trawling disturbances. These findings suggest the relative stability of sensitivity values for species strongly responding to pressure as their abundance decreases considerably along the anthropogenic disturbance gradient. In this case, the addition of samples from the disturbed (low ES50) sites into analysis with possibly high number of zero abundance cases for these species, will typically lead to minor changes of their sensitivity values. In contrast, tolerant species are persistently abundant across the disturbance gradient and are typically present at the intermediate levels of the impact; therefore, an addition of samples from these areas may significantly alter the sensitivity values (Fig. 23). For example, the results for eutrophication disturbance in the coastal waters showed that, more than 40% of species did not change sensitivity values when samples from the impacted sites were included, while at the dredge spoil dumping area this proportion was 25% and at the bottom trawling area the species sensitivity values did not change for 33% of macrozoobenthos species. Generally, the outcome of sensitivity valuation of tolerant species appears to be highly dependent on the coverage of the disturbance gradient by the dataset, while sensitive species are highly robust to this effect.

## 5. Discussion

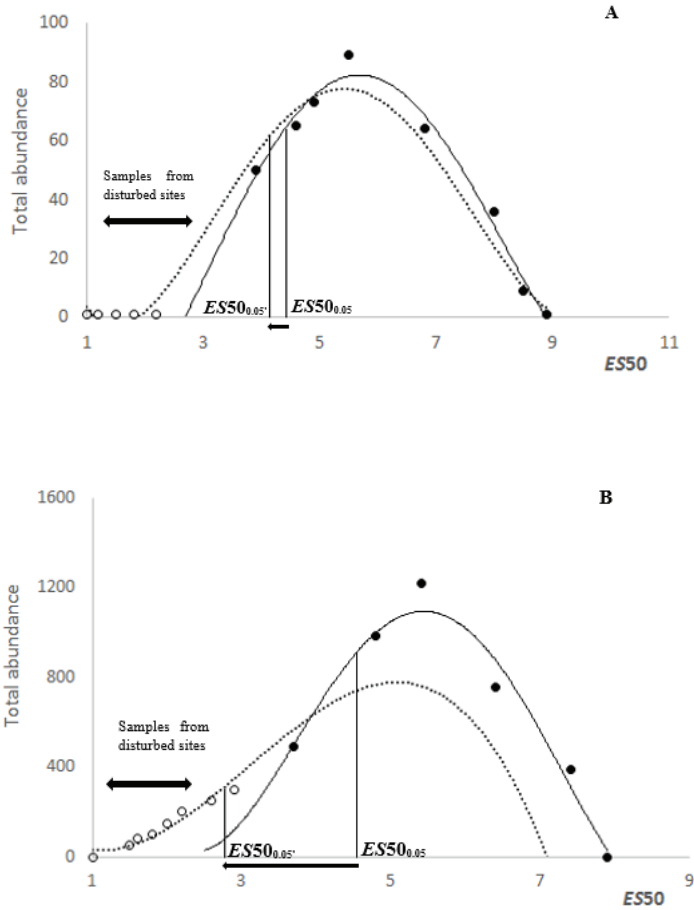


Figure 23. Simulation of sensitivity shift scenarios with: (A) addition of samples from disturbed sites with zero density (open symbols) of sensitive species resulting in minor change of its sensitivity value ( $ES50_{0.05'}$ - $ES50_{0.05}$ ); (B) addition of samples from disturbed sites with decreasing density of tolerant (open symbols) species resulting in clear sensitivity shift ( $ES50_{0.05'}$ - $ES50_{0.05}$ ).

23 paveikslas. Makrozoobentos rūšių jautrumo verčių pokyčio scenarijai: (A) jautrių rūšių atveju, kai tokios rūšys išnyksta esant santykinai mažam poveikio intensyvumui ( $ES50_{0.05'}$ - $ES50_{0.05}$ ); (B) tolerantiškų rūšių atveju, kai tokių rūšių gausumas mažėja esant santykinai intensyviai poveikiui ( $ES50_{0.05'}$ - $ES50_{0.05}$ ).



Estimating the role of varying sensitivity values in the assessment, BQI values can be up to 60-100% higher for species sensitivity values based on limited disturbance gradient. Although this is the upper range of deviation found in the study with the bottom trawling disturbance gradient, it obviously shows the need of having adequate species response data to a given disturbance when defining sensitivity groups and sensitivity values. On the other hand, in spite of a relatively large effect of sensitivity estimates on BQI values, the impact of varying sensitivity values on discrimination between the disturbed and undisturbed samples was negligible. This demonstrates that considerable shift in BQI values is likely to be of a systematic nature and in agreement with a proposal to use a regression model to account for the depth effect and remove much of the spatial variation (Leonardsson et al., 2016).

### 5.3 Dependence of species sensitivity values and Benthic Quality Index values on disturbance type

It was shown that BQI values respond to organic load, hypoxia, heavy metals, urban effluents and physical disturbance (Josefson et al., 2009). Gislason et al. (2017) also demonstrated that BQI respond significantly to the bottom trawlings effects. The BQI response was highly significant, caused by a combination of declines in the average species sensitivity values and in the number of species recorded per station (Gislason et al., 2017). The response of the macrozoobenthos species may differ considerably depending on type of anthropogenic pressure such as eutrophication, organic enrichment, chemical pollution or mobile bottom-contacting fishing gears (Kaiser et al., 2006; Clark et al., 2016; Neumann et al., 2016; Collie et al., 2017), therefore species sensitivity values and indexes may also depend on analysed disturbance type (Rönnberg and Bonsdorff, 2004).

In this study three types of anthropogenic disturbances (bottom trawling, eutrophication and dredge spoil dumping) were analysed in geographically close sites with the distance of approximately 40-50 km between the areas. Eutrophication is spatially more widespread pressure acting over longer time scales in the central Baltic compared to local and relatively short-time dredge spoil dumping and bottom trawling effects. After the addition of the impacted samples to the datasets of samples from the undisturbed sites, the sensitivity values were modified by 4-57% (19% on average) under eutrophication effect, 29-50% (24% on average) under bottom trawling disturbance, and 2-13% (5% on average) under dredge spoil dumping. In the context of these changes, the differences in environmental settings of three areas should be taken into account and can be screened at least partly by comparison of sensitivities estimated from the control datasets of the same areas. According to this comparison, the sensitivity values between the three areas differ by a factor of 2.0-5.8 for individual

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species. On the other hand, the samples from the shallow eutrophication area shared only 17-33% of macrozoobenthos species with deeper areas of dredge spoil dumping and bottom trawling effects. Although two deeper areas shared 62-83% of macrozoobenthos species, the control samples from the bottom trawling area contained only 2-6 species versus 6-13 taxa found in both dredge spoil dumping and coastal eutrophication areas.

According to the study results, the species being classified at higher sensitivity range in the eutrophication and dredge spoil dumping areas obtained the lowest sensitivity values in the deeper areas of the bottom trawling zone. For instance, in the depth range between 40 and 65 m polychaete *B. sarsi* was among the most sensitive species in the dredge spoil dumping area and at the same time had the lowest sensitivity values in the bottom trawling zone. Also, another polychaete species *Marenzelleria* spp. showed similar results. It was one of the sensitive species in the coastal waters area under eutrophication disturbance, while had one of the lowest sensitivity values in the dredge spoil dumping and bottom trawling zones. This pattern remained if control samples only were taken into account, discarding the possibility of the species' specific response to the three considered disturbances.

Other factors, such as species motility or presence of pelagic development stages can significantly decrease the sensitivity values of species due to their higher recolonization capacity. In these cases, although being sensitive to the disturbance impact, the species can be among the first recolonisers of the disturbed samples during early recovery stages and these recolonizations are particularly relevant for patchy or short-term disturbance effects such as bottom trawling and dredge spoil dumping. In this study such effects can be valid for bottom trawling resistant polychaete *B. sarsi*, which is a fast colonizer in intermittently recovering areas usually occurring in low oxygen deep water parts of the Baltic Sea and the same is valid for priapulid *H. spinulosus* with its exceptionally high resistance to anoxia (Janssen and Oeschger, 1992; Oeschger and Vetter, 1992). Although a part of the sensitivity values change was attributed to the disturbance type, it is obvious that altered environmental conditions (specifically associated with the depth or community structure) rather than the type of disturbance was primarily linked to the changes of sensitivity values. Depth-dependent structural community differences obviously played a more important role in the assessment of species sensitivity values, than the disturbance type and coverage of disturbance gradient by samples (e.g. determination of the control and impacted samples according to the macrozoobenthos abundance).

### 5.4 Benthic Quality Index values validation using Signal detection theory method

The study results support the applicability of the BQI values for benthic quality assessment in relation to eutrophication in the exposed coastal waters area. In the Baltic Sea, eutrophication constitutes one of the most important pressures affecting different ecosystem components-from phytoplankton to the macrozoobenthos 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, Chl-*a*, nutrient concentrations and others (Ferreira et al., 2011). 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, which can be also partly traced in the dynamics of Chl-*a*. During succession, this phytoplankton biomass turns into organic material and becomes a food source for the macrozoobenthos in case material transfer to the near-bottom layer or sediment is supported by vertical flux.

Macrozoobenthic diversity explains the nutrient-invertebrate relationships best. The previous studies showed that the impact of macrozoobenthos on benthic-pelagic coupling (sedimentation, particle uptake), benthic mineralisation, nutrient and dissolved organic matter release in shallow water appears to be considerable and disappearance of macrozoobenthos has a significant influence on shallow water energy and matter cycles (Heip, 1995). It was also shown that the response of macrozoobenthos to the changes in total nitrogen concentration decreased with increasing depth. It was demonstrated that the relationships between macrozoobenthos biomass and nutrients (TN and TP) were little affected by coastal water exposure (Kotta et al., 2009; Rousi et al., 2013). It was found that the deposit feeders, were less sensitive to the increased concentration of nutrients if inhabiting more diverse communities. 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 (e.g. changed reproduction rate, modified feeding activity, increased physiological stress etc.) (Heip, 1995; Grall and Chauvaud, 2002; Kotta et al., 2009). As a result, a certain lag period is typically involved in the relationships between benthic and pelagic parameters (e.g. Snickars et al., 2014).

The BQI values response to the analysed eutrophication parameters was not detected (except for Chl-*a*) with the traditional statistical approach (linear regression), while the more complex SDT proved to be effective in uncovering these relationships in this study. However, when applying SDT we found inconsistencies in the BQI values response to Chl-*a* and TP within the plume zone. Stronger BQI values response to TN compared to TP driven by organic matter production is more likely in

the plumes, since highly eutrophied areas with a low-salinity regime are known to be predominantly N-limited (Tamminen et al., 2007). On the other hand, strong spatial and temporal salinity effects may not necessarily coincide with organic enrichment gradients in the plume zone.

Low salinity has been reported as the driving force of the macrozoobenthos distribution in many estuarine-like ecosystems (e.g. Boesch, 1977; Ysebaert et al., 1993; Bonsdorff, 2006), where its effect was asserting independently from the influence of organic enrichment (mud content, Chl-*a*). It is obvious, that even being of the same resistance to organic enrichment, different species may have individual salinity tolerances, and this may become particularly important in the plume areas with transition between freshwater and the critical salinity range of 5-8. At the same time, testing ability of BQI to discriminate between salinity and eutrophication effects will require highly specific conditions with low cross-correlation of key parameters.

The overarching purpose of any environmental index 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 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 index thresholds and select good environment status (GES) boundary for a particular area. Based on the SDT analysis, one could decide whether an index 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.

### 5.5 Recommendations for the water quality status assessment of the south-eastern Baltic Sea using Benthic Quality Index

The following recommendations are provided for the water quality status assessment of the south-eastern Baltic Sea using the BQI based on the results of this thesis:

1. Water quality assesment using BQI should be carried out using justified species sensitivity values for the assessed macrozoobenthos communities, depth zones and salinity range. All relevant environmental parameters of the assessment area used in structuring datasets should be precisely described and justified.

1.1 Species recorded in 8 samples or less should be excluded from further species sensitivity values determination. Only soft bottom species should be present in the sample.

1.2 Below 40 m depth typical shallow species (such as *C. glaucum*, *S. shrubsolii*, *B. pilosa*, *M. arenaria*, *C. volutator*, *H. diversicolor*, *Hydrobia* sp.) should not be included in the assessment dataset.

1.3 For the coastal waters with the depth less than 40 m “deeper living” species (such as *B. sarsi*, *H. spinulosus*, *S. entomon*, *M. affinis*, *Ostracoda* undet.) should be excluded from the assessment dataset.

1.4 Dominant macrozoobenthos communities should be distinguished according to the species abundance, biomass, bottom sediment and other parameters data before using dataset for the water quality assessment.

2. Species sensitivity values used in the water quality assesment with BQI should be justified for the expected types of anthropogenic disturbance and justification should be scientifically documented in order to support the correct interpretation of assessment results.

2.1 Species sensitivity values should be estimated for as wide as possible disturbance gradient keeping at least 20 samples from each of control and impacted areas in order to track the precise detection limits of the index.

2.2 In order to cover wider range of a species response along the disturbance gradient, species sensitivity values can be estimated for samples with less than 50 individuals. The validity of samples with a low number of individuals should be justified by the identical *ES50* values and number of species for corresponding samples, therefore the original *ES50* values could be used for further sensitivity calculations in order to extend the representation of disturbance gradient by the dataset.

2.3 At the eutrophication area an impacted samples could be characterised according to the spartial sattelite Chl-*a* data (increase of the concentration, one-year lag should be applied for the index values in respect of pelagic parameters); at the dredge spoil dumping area- according to the designated dumping sites, indicator species such as *P. elegans* or *Ostracoda* undet. abundance change, bottom sediment data, while at the bottom trawling zones- according to the information on sampling site position in

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respect to acoustically mapped bottom trawling tracks should be used as criteria to justify bottom trawling effects. Also evaluated species communities should be characterised according to the species structure.

3. Four sensitivity groups of macrozoobenthos species can be defined by dividing sensitivity range into equal intervals to have a feasible response to the BQI change.

3.1 Numerical characteristics of macrozoobenthos sensitivity groups are useful in the interpretation of water quality class boundaries and quality assessment results, however, these values should be statistically validated.

4. The one-year lag proved to be statistically feasible for the BQI relationship with pelagic parameters, while approximately 3x3 km<sup>2</sup> grid for eutrophication parameters provided statistically valid results for explaining the BQI changes.

4.1 The Signal detection theory method is suggested for BQI validation as well as determination of index values for water quality class boundaries and justification of their selection strategy between lenient (maximum NPV and index sensitivity) and strict (maximum PPV and index specificity) values.

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## Conclusions

1. The soft-bottom macrozoobenthos species sensitivity values are highly dependent on the depth gradient. In the depths between 10 and 70 m the sensitivity values were consistent only for the most widespread 4 taxa (*Marenzelleria* spp., *P. elegans*, *L. balthica*, *Oligochaeta* undet.) out of 16 analysed species. The critical depth of 30-40 m identified for both groups of shallow (e.g. *C. glaucum*) and deeper (e.g. *S. entomon*) living species indicate potentially over-estimated species sensitivity values. For all the analysed the species sensitivity values decrease towards the upper boundary of the halocline zone (50 m).
2. The consistency analysis of the species sensitivity values and Benthic Quality Index values showed a significant difference between the soft bottom communities dominated by bivalve *L. balthica* and polychaete *Marenzelleria* spp. within the same depth range. The species diversity and abundance in *Marenzelleria* spp. community was lower compared to *L. balthica* community and this resulted in up to two times lower sensitivity values and Benthic Quality Index values in polychaete dominated areas.
3. The sensitivity values of tolerant species are highly dependent on the coverage of anthropogenic disturbance gradient by the data, while sensitive species are robust to this effect.

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4. Different anthropogenic impacts have specific sets of tolerant and sensitive species in tracing effects on environmental status. Eutrophication effects are best reflected by bivalve *M. arenaria*, *C. glaucum*, *Hydrobia* sp. and amphipod *B. pilosa*, whereas tracing of dredge spoil dumping and bottom trawling disturbance relies more on the dynamics of *P. elegans*-ostracods and *Marenzelleria* spp.-ostracods, respectively. Similarly, a set of tolerant species also differ between the analysed disturbance types, but *L. balthica* was found to be tolerant to all three analysed disturbance types.
5. The Signal detection theory method proved to be useful in the index validation and justification of quality class boundaries. In contrast to the Benthic Quality Index values validation by linear regression, the Signal detection theory indicated an acceptable Benthic Quality Index values response to the total nitrogen, total phosphorus and chlorophyll-a concentration for the studied coastal waters.



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## Santrauka

### Įvadas

#### Temos aktualumas

Makrozoobentosas seniai naudojamas jūrinių ekosistemų būklei vertinti (Borja ir kt. 2000), o makrozoobentos rodikliais pagrįsti indeksai yra privalomi vertinant jūrų bei priekrantės vandenų būklę pagal naujausias aplinkosaugines Europos Sąjungos iniciatyvas: Europos Sąjungos Vandens politikos (2000/60/EC) ir Jūros strategijos pagrindų direktyvas (2008/56/EC).

Rūšies jautrumą lemia ne tik jos biologinės savybės, bet ir gamtinės sąlygos, kur rūšys gali skirtingai reaguoti į aplinkos (Tagliapietra ir kt. 2009) ar antropogeninius pokyčius (Buhl-Mortensen ir kt. 2009). Makrozoobentos rūšies jautrumas apibūdinamas kaip galimos žalos dėl atsiradusio poveikio (atsparumas) ir laiko, kurio prireikia atsigausti, kai poveikio nebėra (atsistatymas), produktas (Laffoley ir kt. 2000). Jautriomis rūšimis laikomos tokios rūšys, kurioms lengvai daro įtaką antropogeninis poveikis (žemas atsparumas) ir kurios atsigauna tik po ilgesnio laiko arba visai neatsigauna (žemas atsistatymas). Makrozoobentos rūšių jautrumas yra viena svarbiausių būklės indeksų sudedamoji dalis, kuriai apibūdinti dažniausiai naudojamas ekspertinis vertinimas (angl. The AZTI Marine Biotic Index; Borja ir kt. 2000; angl. The Biotic Index; Simboursa

ir Zenetos 2002). Tačiau tokiu būdu sudėtinga matematiškai įvertinti rūšių atsparumą, atkuriamumą ir pažeidžiamumą. Ekspertiniu vertinimu nustatytos rūšių jautrumo vertės gali turėti fiksuotą verčių kitimą, iš anksto nustatytas jautrumo klases ir fiksuotą atstumą tarp skirtingų jautrumo klasių, kurioms priskirti organizmai. Šios savybės lemia paprastą rūšių jautrumo verčių apskaičiavimą ir interpretavimą vertinant vandens būklę.

Bentoso kokybės indekso (angl. Benthic Quality Index, BQI) (Rosenberg ir kt. 2004; Leonardsson ir kt. 2009) viena iš svarbiausių sudedamųjų dalių – rūšies jautrumas, kuris nustatomas skaitiniais metodais, nesikliaujant ekspertiniu vertinimu. Baltijos jūros pavyzdžiu jau parodyta, kad makrozoobentoso rūšių jautrumo vertės priklauso nuo vandens druskingumo (Zettler 2007), organinės medžiagos kiekio nuosėdose (Zettler ir kt. 2013) ar invazinių rūšių buvimo (Zaiko ir Daunys 2015). Bentoso rūšių jautrumo vertės buvo skaičiuojamos skirtinguose Baltijos jūros regionuose (Rosenberg ir kt. 2004; Zettler 2007) skirstant juos pagal druskingumą, gylio klases ir mėginių surinkimo įrankių tipus (Schiele ir kt. 2016).

Šių studijų tikslas buvo patikrinti makrozoobentoso rūšių jautrumo verčių patikimumą keičiantis gylio gradientui, bendrųjų struktūrai ir antropogeninio poveikio tipui bei lygiui, kur bentoso kokybės indekso vertės skirtingai reagavo į tirtus parametrus. Taip pat indekso specifiškumas ir jautrumas buvo testuojamas naudojant Signalo aptikimo teorijos metodą, vertinant indekso atsaką į pasirinktus eutrofikacijos procesą apibūdinančius parametrus. Validuotos indekso vertės panaudotos vandens būklės slenkstinėms vertėms nustatyti. Pateiktos indekso naudojimo rekomendacijos prietrytinėje Baltijos jūros dalyje vandens būklei vertinti.

### Tyrimo tikslas ir pagrindiniai uždaviniai

Studijų tikslas – įvertinti makrozoobentoso rūšių jautrumo verčių pokyčius ir jų įtaką bentoso kokybės indekso pritaikomumui, vertinant ekosistemų būklę mažos biologinės įvairovės bendrijose pietrytinėje Baltijos jūros dalyje.

Darbo tikslui pasiekti buvo iškelti šie uždaviniai:

1. įvertinti vandens gylio svarbą makrozoobentoso rūšių jautrumo vertėms ir jų panaudojimą skaičiuojant bentoso kokybės indeksą;
2. kiekybiškai įvertinti makrozoobentoso rūšių jautrumo ir bentoso kokybės indekso verčių pokyčius keičiantis bendrųjų struktūrai;
3. įvertinti rūšių jautrumo verčių patikimumą skirtingai antropogeninei veiklai ir poveikio lygiui;
4. pritaikyti ir testuoti Signalo aptikimo teorijos metodą validuojant bentoso kokybės indekso vertes bei nustatant vandens būklės klasių slenkstines vertes;
5. parengti vandens būklės vertinimo rekomendacijas, kaip vertinti pietrytinės Baltijos jūros dalies vandens ekosistemų būklę panaudojant bentoso kokybės indeksą.

### Darbo naujumas

Kaip tyrimo rezultatai pristatomi makrozoobentos rūšių jautrumo verčių pokyčiai keičiantis skirtingoms antropogeninėms veikloms, gyliui ir bendrųjų struktūroms bei šių faktorių įtaka galutinėms bentos kokybės indekso vertėms ir vandens būklės vertinimui. Tyrimas atliktas pietrytinėje Baltijos jūros dalyje, ir jis patvirtino rezultatus, būdingus visam Baltijos jūros regionui – nustatytą jautrumo verčių priklausomybę nuo gylio (Schiele ir kt. 2016; Leonardsson ir kt. 2016). Pirmą kartą atlikta Baltijos jūros makrozoobentos rūšių jautrumo verčių išsami analizė, tiriant dvi dominuojančias bendrijas tame pačiame gylyje, ir ji parodė reikšmingus jautrumo verčių pokyčius. Taip pat tyrimo metu bentos kokybės indekso vertės buvo validuotos panaudojant Signalo aptikimo teorijos metodą, kuris leidžia nustatyti indekso atsaką į pasirinktus parametrus, nustatyti vandens būklės klasių slenkstines vertes. Bentos kokybės indeksas buvo testuotas pritaikius aštuonių kokybės kriterijų vertinimo sistemą, ir rezultatai leido įvertinti šį indeksą kaip gerą indikatorių vertinant Bioįvairovės (D1) ir Jūros dugno vientisumo (D6) deskriptorius.

### Rezultatų mokslinė ir praktinė reikšmė

Svarbiausia mokslinė šio darbo prasmė yra išsami makrozoobentos rūšių jautrumo verčių patikimumo ir tikslumo analizė naudojant pastovius aplinkos ilgalaikio monitoringo ir trumpų tyrimo projektų duomenis. Tyrimo rezultatai suteikia žinių apie vandens būklės vertinimą pietrytinėje Baltijos jūros dalyje panaudojant bentos kokybės indeksą ir leidžia pateikti detalias rekomendacijas. Šios žinios buvo pritaikytos vertinant vandens būklę ir nustatant vandens klasių slenkstines vertes priekrantėje, šios slenkstinės vertės buvo patvirtintos Lietuvos Respublikos aplinkos ministerijoje vykdam Vandens politikos bei Jūros strategijos pagrindų direktyvas.

### Ginamieji teiginiai

1. Makrozoobentos rūšių jautrumo vertės atspindi rūšių atsaką į natūralius pokyčius ir antropogeninės veiklos poveikius, tačiau bentos kokybės indekso vertės vertinant vandens būklę turi būti taikomos tiksliai apibūdinant aplinkos būklę, bendrijos struktūrą ir esamus poveikius.

2. Jautrios ir tolerantiškos makrozoobentos rūšys skirtingai reaguoja į antropogeninės veiklos poveikius, ir rūšių jautrumo vertės yra priklausomos nuo duomenų, surinktų iš poveikio vietų, kokybės.

3. Signalo aptikimo teorijos metodas gali būti naudojamas bentos kokybės indekso verčių validacijai, pasirenkant poveikio parametrus, ir vandens būklės klasių slenkstinėms vertėms nustatyti.

### Rezultatų apibavimas

Šio darbo rezultatai buvo pristatyti trijose tarptautinėse ir dviejose nacionalinėse konferencijose.

ECSA 56 mokslinė konferencija Priekrantės sistemos būklė pereinamuoju laikotarpiu: nuo „natūralių“ iki „paveiktų antropogeninio poveikio“, Brėmenas, Vokietija, rugsėjis, 2016 m.

Mokslinis simpoziumas 2015: „Europos vandenų ekosistemos vertinimo būdai“, Malmė, Švedija, gegužė, 2015 m.

10-asis Baltijos jūros mokslų kongresas, Ryga, Latvija, birželis, 2015 m.

9-oji mokslinė-praktinė konferencija „Jūros ir krantų tyrimai“, Klaipėda, Lietuva, balandis, 2016 m.

10-oji mokslinė-praktinė konferencija „Jūros ir krantų tyrimai“, Palanga, Lietuva, balandis, 2017 m.

Šios disertacijos rezultatai buvo paskelbti mokslinėse publikacijose:

**Chušėvė R.**, Nygård H., Vaičiūtė D., Daunys D., Zaiko A. (2016) Application of signal detection theory approach for setting thresholds in benthic quality assessments. *Ecological Indicators* 60, 420–427.

Queiros A. M., Strong J. A., Mazik K., Carstensen J., Bruun J., Somerfield P. J., Bruhn A., Ciavatta S., **Chušėvė R.**, Nygård H., Flo E., Bizsel N., Ozaydinli M., Muxika I., Papadopoulou N., Pantazi M., Krause-Jensen D. (2016) An objective framework to test the quality of candidate indicators of good environmental status. *Frontiers in Marine Science*. 10.3389/fmars.2016.00073.

**Chušėvė R.**, Daunys D. (2017) Can benthic quality assessment be impaired by uncertain species sensitivities? *Marine Pollution Bulletin*. 116, 332–339.

### Disertacijos struktūra

Disertaciją sudaro šie skyriai: Įvadas, Literatūros apžvalga, Medžiaga ir metodai, Rezultatai, Diskusija, Išvados, Literatūros sąrašas. Disertacijos apimtis – 86 puslapiai. Disertacijoje panaudota 119 literatūros šaltinių. Disertacija parašyta anglų kalba. Joje yra 13 lentelių ir 23 paveikslai.

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## TYRIMŲ MEDŽIAGA IR METODAI

### Tyrimų rajonas

Tyrimai buvo atliekami atviroje pietrytinėje Baltijos jūros dalyje ir Lietuvos priekrantėje. Studijų metu buvo analizuojamos Lietuvos vandenyje vykdomos antropogeninės veiklos: eutrofikacija, grunto pylimas ir dugninis tralavimas (3 pav.).

### Duomenų rinkimas

Makrozoobentos rūšių jautrumo ir bentoso kokybės indekso verčių analizei buvo panaudoti ilgamečiai ir trumpalaikių projektų metu surinkti makrozoobentos gausumo ir rūšinės sudėties duomenys, apimantys laikotarpį nuo 2002 m. iki 2015 m. (1 lent.), iš kurių sudaryti 8 duomenų rinkiniai, naudoti analizėms (2 lent.). Mėginiai buvo renkami minkštuose gruntuose, 10–70 m gylyje Van Veen gruntotraukiu (0,1 m<sup>2</sup>), turinį plaunant pro 0,5 mm akies dydžio sieta, ir laboratorijoje analizuoti pagal HELCOM rekomendacijas (HELCOM, 2012). Validacijai buvo naudoti satelitiniai Chl-*a* bei ilgamečio monitoringo metu rinkti bendro azoto ir fosforo koncentracijos duomenys.

### Duomenų rinkinių sudarymas ir statistinė analizė

Duomenų analizei naudoti parametriniai (Stjudento t-testas) ir neparametriniai (Kruskalo-Voleso, Mano-Vitnio testai) metodai (R-project, 2014 statistinė programa). Vilkoksono rangų ženklų testas naudotas normališkumui (Shapiro-Vilko testas) ir heteromogeniškumui nustatyti (Levene testas) (Statistinis paketas socialiniams mokslams (SPSS), 19 versija, 2010). Naudojantis R v3 statistine programa (R-project, 2014 statis-

tinė programa, R programos skriptas sukurtas A. Darr) buvo skaičiuojamos kiekvieno mėginio Hurlberto įvairovės indekso ir bentoso kokybės indekso vertės, o kiekvienai makrozoobentoso rūšiai – jautrumo vertės (Rosenberg ir kt. 2004; Leonardsson ir kt. 2009).

Remiantis makrozoobentoso rūšine struktūra, rūšių paplitimo ribomis, sutinkamumu, gausumo charakteristikomis (nMDS ordinacija, angl. non-metric multidimensional scaling, nMDS, procentinė panašumo, angl. similarity percentages, SIMPER ir panašumo analizė, angl. analysis of similarities, ANOSIM) analizuojamas gylio intervalas (10–70 m) buvo suskirstytas į 10–30 m, 30–50 m, 50–70 m intervalus, ir naudojant šiuos duomenų rinkinius atskiriems gylio intervalams ir visam gylio intervalui (10–70 m) buvo paskaičiuotos rūšių jautrumo ir bentoso kokybės indekso vertės. Indekso vertės, gautos panaudojus rūšių jautrumo vertes iš skirtingų vandens gylio intervalų, buvo palygintos su indekso vertėmis, kurioms apskaičiuoti panaudotos jautrumo vertės iš viso gylio intervalo (10–70 m) (1 pav., 2 lent.).

Bendrijų rūšinės sudėties poveikio rūšių jautrumo verčių analizei naudoti dviejų priekrantės (iki 30 m) dominuojančių makrozoobentoso bendrijų duomenys – dvigeldžio moliusko *Limecola balthica* (n=54), daugiašerės kirmėlės *Marenzelleria* spp. (n=51) ir neišskyrus bendrijų (bendras duomenų rinkinys) (2 pav., 2 lent.). Bentoso kokybės indekso vertės, kurioms apskaičiuoti buvo naudoti atskirų bendrijų duomenų rinkiniai, buvo palygintos su vertėmis, kurioms gauti buvo naudotos rūšių jautrumo vertės iš bendro duomenų rinkinio.

Antropogeninio poveikio analizei rūšių jautrumo ir bentoso kokybės indekso vertės apskaičiuoti buvo naudoti eutrofikacijos poveikio (10–30 m, n=65), grunto pylimo (40–50 m gyliai, n=118), dugninio tralavimo (60–65 m gyliai, n=48) rajonuose surinktų mėginių informacija – makrozoobentoso rūšių gausumai ir įvairovė (3 pav., 2 lent.).

Bentoso kokybės indekso vertės priekrantėje buvo validuotos ir vandens būklės klasės slenkstinės vertės nustatytos pagal indekso verčių ir makrozoobentoso rūšių jautrumo grupių kaitą (2 pav., 2 lent.). Šios grupės charakterizuotos padalinus apskaičiuotą rūšių jautrumo verčių intervalą į keturias lygias dalis (Osowiecki ir kt. 2008, Leonardson ir kt. 2009; Fleischer ir Zettler 2009). Validuojant bentoso kokybės indekso vertes, makrozoobentoso rūšys pagal jautrumo reikšmių intervalus buvo charakterizuotos kaip labai jautrios, jautrios, tolerantiškos ir labai tolerantiškos.

Bentoso kokybės indekso vertės buvo validuotos su eutrofikacijos procesą apibūdinančiais parametrais (Chl-*a*, bendra fosforo ir azoto koncentracijomis) priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje (2 pav., 2 lent.). Chl-*a* vertės buvo apskaičiuotos kiekvienos bentoso stebėjimų vietos aplinkiniam rajonui (apytiksliai 3x3 km<sup>2</sup>). Validuojant indekso vertes, parametrai poruoti su metais vėlesnėmis bentoso kokybės indekso vertėmis tokiu būdu taikant prielaidą, kad padidėjusios fitoplanktono produkcijos poveikis dugno makrozoobentoso struktūrai registruojamas metais vėliau.

Geros–vidutinės vandens būklės klasės indekso slenkstinė vertė buvo nustatyta naudojantis Signalo aptikimo teorijos metodu. Šio tyrimo metu indeksas buvo testuo-



jamas su eutrofikacijos procesą apibūdinančiais parametrais, kur geros–vidutinės vandens būklės klasės slenkstinės vertės nustatytos pagal Vandens politikos direktyvos Lietuvos paviršinio vandens vertinimo metodologiją (Langas ir kt. 2009). Priėmimo charakteristikos kreivės (angl. Receiver Operating Characteristic, ROC) buvo naudojamos grafiškai atvaizduoti indekso specifiškumą ir jautrumą pasirinktam testuojamam eutrofikacijos procesą apibūdinančiam aplinkos parametrai.

## REZULTATAI

Rezultatai pristatyti keturiuose skyriuose: 1) Makrozoobentosos charakteristikos keičiantis gylio gradientui; 2) Rūšių jautrumo verčių patikimumo vertinimas; 3) Bentoso kokybės indekso verčių vertinimas; 4) Bentoso kokybės indekso verčių validacija.

**Pirmame skyriuje** pateikiami rezultatai, apimantys makrozoobentosos charakteristikų analizę pagal gylio gradientą. Iš viso identifikuota 24 makrozoobentosos minkšto dugno rūšių ar aukštesnių taksonų, tačiau dėl duomenų patikimumo rūšių jautrumo vertės buvo apskaičiuotos 16 rūšių (4 lent.). Pagal makrozoobentosos struktūros analizės 10–70 m gylio intervaluose rezultatus buvo išskirtos 3 rūšių grupės. Pirmoje grupėje buvo identifikuotos 7 makrozoobentosos rūšys, kurių pusė buvo paplitusios 20–30 m gylyje, o kitos rūšys pasitaikė / buvo giliau negu 40 m. Dvigeldžių moliuskų *Cerastoderma glaucum*, *Mya arenaria* ir daugiašerės kirmėlės *Hediste diversicolor* rūšių sutinkamumas buvo vienas didžiausių tarp visų tirtų sėkliausių rūšių, tačiau visų šios grupės rūšių sutinkamumas mažėjo didėjant gyliui, ypač 30–40 m gylio intervale (4 lent.). Antrai makrozoobentosos rūšių grupei buvo priskirtos plačiai paplitusios visame gylio gradientu rūšys, tokios kaip mažiašerės ir daugiašerės kirmėlės *Oligochaeta* undet., *P. elegans*, *Marenzelleria* spp. bei dvigeldis moliuskas *L. balthica*. Trečiai makrozoobentosos grupei buvo priskirtos giliau vandens rūšys (daugiašerė kirmėlė *Bylgides sarsi*, priapulidas *Halicryptus spinulosus*, šoniplauka *Monoporeia affinis*, kiaušavėžis *Ostracoda* undet. ir lygiakojis *Saduria entomon*), kurios paplitusios giliau negu 30 m.

Atlikus makrozoobentosos gausumo duomenų analizę paaiškėjo, kad, neskaitant per visą gylio gradientą paplitusių rūšių, 10–30 ir 50–70 m gylio intervaluose makrozoobentosos rūšinės sudėties ir gausumo grupės skiriasi. Didelis taksonominis rūšių struktūros pokytis 30–40 m gylyje sutapo su rūšių gausumo pokyčiais 30–50 m gylyje, todėl visas analizuojamas gylio gradientas (10–70 m) tolimesnei rūšių jautrumo verčių pokyčių analizei padalintas į sekliąją – iki 30 m, giliają – giliau 50 m ir viduriniąją (30–50 m) dalis.

**Antrame skyriuje**, kuris suskirstytas į tris poskyrius, pateikti makrozoobentosos rūšių jautrumo verčių, apskaičiuotų skirtinguose gyliuose, dominuojančiose makrozoobentosos bendrijose bei esant skirtingam antropogeniniam poveikio tipui ir lygiui, rezultatai.

**Pirmame poskyryje** pateikiama gylio poveikio rūšių jautrumo vertėms analizė, kur jautrumo vertės apskaičiuotos iš skirtingų gylio intervalų (10–30 m, 30–50 m, 50–70 m) ir visame gylio gradiente (10–70 m). Tyrimo metu nebuvo rasta makrozoobentosos rūšių, turinčių pastovias jautrumo vertes keičiant duomenų rinkinius, kita vertus tik 4 iš 16 analizuotų rūšių buvo rastos visame gylio gradiente (10–70 m). Mažiausios jautrumo vertės buvo rastos spionidams *P. elegans*, *Marenzelleria* spp., mažašerėms kirmėlėms (*Oligochaeta* undet.) ir dvigeldžiui moliuskui *L. balthica* (5 lent.). Giliau negu 50 m rūšių jautrumo vertės mažėjo mažėjant gausumui, tačiau daugelis (išskyrus mažašeres kirmėles) rūšių išliko gausiausios. Dvigeldžių moliuskų *M. arenaria*, *C. glaucum*, daugiašerės kirmėlės *H. diversicolor* ir vėžiagyvio *C. volutator* jautrumo vertės apskaičiuotos iš 10–30 m gylio intervalo didėjo palyginus su reikšmėmis, gautomis iš 30–50 m intervalo, tačiau šių rūšių gausumas keičiantis gyliui mažėjo. Dvi sėkliamėgės rūšys, kaip šoniplauka *Bathyporeia pilosa* ir daugiašerė kirmėlė *Streblospio shrubsolii*, buvo retos ir negausiai paplitusios tik 10–30 m gylyje. Giliavandenių rūšių (kiautavėžių *Ostracoda* undet., priapulido *H. spinulosus*, šoniplaukos *M. affinis*, daugiašerės kirmėlės *B. sarsi*, lygiakojo *S. entomon*) jautrumo vertės ir gausumas mažėjo didėjant gyliui (palyginus vertes iš 30–50 m ir 50–70 m gylio intervalų). 50–70 m gylyje mažiausios jautrumo vertės buvo apskaičiuotos *Marenzelleria* spp. ir *B. sarsi* rūšims, o didžiausios vertės buvo apskaičiuotos *Ostracoda* undet. ir *M. affinis* rūšims (5 lent.).

**Antrame poskyryje** pateikiama rūšių jautrumo verčių rezultatų analizė dviejose dominuojančiose bendrijose – dvigeldžio moliusko *L. balthica* ir daugiašerės kirmėlės *Marenzelleria* spp. bei jautrumo verčių apskaičiavimas neišskyrus bendrijų (bendras duomenų rinkinys) (6 lent.). Tirtose makrozoobentosos bendrijose rūšių jautrumo vertės statistiškai reikšmingai skyrėsi (Mano-Vitnio testas,  $p=0,0001$ ), kur verčių intervalas kito nuo 4,1–5,2 *L. balthica* bendrijoje bei 1,9–3,9 *Marenzelleria* spp. bendrijoje (6 lent.). Abiejose tirtose bendrijose rasta gausiausia *Marenzelleria* spp. rūšis, kuriai apskaičiuota mažiausia jautrumo vertė (4,1 dvigeldžio moliusko *L. balthica* bendrijoje ir 1,9 *Marenzelleria* spp. bendrijoje). Rūšių gausumo ir jautrumo verčių pokyčių tarp analizuojamų bendrijų nebuvo rasta, kur, pavyzdžiui, daugiašerei kirmėlei *H. diversicolor* buvo apskaičiuota viena didžiausių jautrumo verčių (5,2) moliusko *L. balthica* bendrijoje, tačiau viena mažiausių verčių – *Marenzelleria* spp. bendrijoje, o pastarojoje *H. diversicolor* rūšies gausumas buvo apie 10 kartų mažesnis negu *L. balthica* bendrijoje. Vertinant rūšių jautrumo vertes ir gausumo pokyčius, apskaičiuotus iš bendro duomenų rinkinio (neišskiriant bendrijų), buvo aptiktos trys tendencijos. Pirma, daugiašerėms kirmėlėms *Marenzelleria* spp., *P. elegans* ir dvigeldžiui moliuskui *L. balthica* buvo apskaičiuotos panašios jautrumo vertės ir vidutinis gausumas (išskyrus *L. balthica*) tarp bendro duomenų rinkinio (neišskiriant bendrijų) ir *Marenzelleria* spp. bendrijos (6 lent.). Skirtingai, mažašerių kirmėlių *Oligochaeta* undet ir dvigeldžio moliusko *M. arenaria* rūšims jautrumo vertės, apskaičiuotos iš bendro duomenų rinkinio (neišskiriant bendrijų) ir iš *L. balthica* bendrijos, buvo

panašios. Daugiašerės kirmėlės *H. diversicolor* rūšiai buvo apskaičiuotas didžiausias jautrumo verčių skirtumas tarp analizuotų bendrijų, kur jautrumo vertė, gauta iš bendro duomenų rinkinio (neišskiriant bendrijų), buvo lygi tiriamų dominuojančių bendrijų (*L. balthica* ir *Marenzelleria* spp.) aritmetiniam vidurkiui.

**Trečiame poskyryje** pateikiama eutrofikacijos, grunto pylimo ir dugninio tralavimo poveikių analizė bei jų įtaka makrozoobentos rūšių jautrumo vertėms.

**Makrozoobentos rūšių jautrumo eutrofikacijos poveikiui verčių pokyčiai.** Eutrofikacijos poveikio rajone iš viso ištirti 65 mėginiai (10–20 m gylyje), kur identifikuota 12 makrozoobentos rūšių. Remiantis nMDS ordinacijos rezultatais pagal makrozoobentos rūšių gausumo duomenis išskirtos 25 poveikio ir 40 foninių vietų (8 pav.). Rūšių jautrumo verčių intervalas, nustatytas remiantis duomenimis iš bendro (poveikio ir foninių) duomenų rinkinio (2,3–5,8), buvo platesnis negu jautrumo verčių intervalas, apskaičiuotas tik iš foninių vietų duomenų rinkinio (4,3–5,8) (7 lent.). Daugiau negu pusei makrozoobentos rūšių jautrumo vertės buvo mažesnės, kurioms apskaičiuoti naudotas bendras (poveikio ir foninių vietų) duomenų rinkinys (Vilkoksono rangų ženklų testas,  $p=0,018$ ), palyginus su vertėmis, kurios gautos naudojant tik foninių vietų duomenų rinkinį. Keičiant duomenų rinkinius didžiausi jautrumo verčių pokyčiai buvo būdingi daugiašerėms *Marenzelleria* spp., *P. elegans*, *H. diversicolor* kirmėlėms, dvigeldžiui moliuskui *L. balthica* ir vėžiagyviui *C. volutator*, kai tuo tarpu *M. arenaria* rūšiai jautrumo vertės kito mažai (7 lent.).

**Makrozoobentos rūšių jautrumo grunto pylimo poveikiui verčių pokyčiai.** Grunto pylimo rajone nustatyta 16 makrozoobentos rūšių, iš kurių pagal biomasę dominavo moliuskas *L. balthica* (40–50 m gylyje), kur rūšių jautrumo vertės buvo apskaičiuotos 8 rūšims. Naudojant nMDS ordinaciją pagal daugiašerės *P. elegans* kirmėlės reikšmingą gausumo sumažėjimą (Vilkoksono rangų ženklų testas,  $p=0,0001$ ) išskirtos foninės ( $511\pm 424$  ind  $m^{-2}$ ,  $n=37$ ) ir poveikio vietos ( $36\pm 37$  ind  $m^{-2}$ ,  $n=81$ ) (Mano-Vitnio testas,  $p<0,0001$ , 10 pav.). Šešių rūšių jautrumo vertės, apskaičiavus pagal poveikio ir foninių vietų duomenų rinkinius, buvo statistškai reikšmingai mažesnės (Vilkoksono rangų ženklų testas,  $p=0,027$ ) lyginant su jautrumo vertėmis, nustatytomis tik foninėse vietose. Daugiašerės kirmėlės *P. elegans* ir kiautavėžio *Ostracoda* undet. jautrumo vertės nekito keičiantis duomenų rinkiniams. Rūšių jautrumo verčių intervalai, nustatyti foninėms (4,1–4,9) ir visoms tyrimų vietoms (3,7–4,8), buvo panašūs (8 lent.).

**Makrozoobentos rūšių jautrumo dugninio tralavimo poveikiui verčių pokyčiai.** Remiantis nMDS ordinacijos rezultatais pagal makrozoobentos rūšinę sudėtį (buvimas / nebuvimas) išskirtos 27 foninės ir 21 poveikio vietos (60–65 m), kur jautrumo vertės apskaičiuotos šešioms rūšims (9 lent.). Poveikio vietose bendras vidutinis rūšių gausumas ( $74\pm 104$  ind  $m^{-2}$ ) buvo statistškai reikšmingai mažesnis (Mano-Vitnio testas,  $p=0,0001$ ) negu foninėse vietose ( $195\pm 125$  ind  $m^{-2}$ ). Rūšių jautrumo verčių intervalai, nustatyti remiantis duomenimis iš foninių (2,0–3,9) ir visų (poveikio

ir foninių) vietų (1,0–3,5), buvo panašūs, tačiau rūšių jautrumo vertės buvo mažesnės skaičiuojant jas pagal bendrą duomenų rinkinį (poveikio ir foninėms) lyginant su jautrumo vertėmis, nustatytomis tik foninėse vietose. Keičiant duomenų rinkinius, didžiausi jautrumo verčių pokyčiai buvo būdingi šoniplaukos *M. affinis*, daugiašerės kirmėlės *B. sarsi*, dvigeldžio moliusko *L. balthica* ir priapulido *H. spinulosus* rūšims. Skirtingai nuo šių rūšių, kiautavėžiams *Ostracoda* undet. ir daugiašerei kirmelei *Marrenzelleria* spp. jautrumo vertės, keičiant duomenų rinkinius, nepakito (9 lent.).

**Trečiame skyriuje**, kuris sudarytas iš trijų poskyrių, pateikiami rezultatai, kaip keičiasi bentoso kokybės indekso vertės, kurioms paskaičiuoti panaudotos rūšių jautrumo vertės iš skirtingų vandens gylio intervalų, aptinkamų bendrijų struktūrų ir antropogeninės veiklos.

**Pirmame poskyryje** pateikiami gylio poveikio bentoso kokybės indekso verčių rezultatai, kuriems apskaičiuoti buvo naudotos rūšių jautrumo vertės, gautos iš skirtingų gylio intervalų (10–30 m, 30–50 m, 50–70 m). Šie rezultatai palyginti su bentoso kokybės indekso vertėmis, kurioms gauti panaudotos rūšių jautrumo vertės, apskaičiuotos iš 10–70 m gylio intervalo. Stiprus ryšys buvo rastas tarp bentoso kokybės indekso verčių, kurioms paskaičiuoti buvo panaudotos rūšių jautrumo vertės iš 10–70 gylio, palyginus su indekso vertėmis, kurios apskaičiuotos pagal rūšių jautrumo vertes iš 10–30 m ir 30–50 m intervalų (11 pav.). Bentoso kokybės indekso vertės, kurioms paskaičiuoti buvo panaudotos rūšių jautrumo vertės iš 10–70 m gylio, kito nuo 3,0 iki 5,1 (vidutiniškai  $4,1 \pm 0,5$ ) ir buvo statistiškai reikšmingai mažesnės (Vilkoksono rangų ženklų testas,  $p < 0,001$ ,  $n = 49$ ) už bentoso kokybės indekso vertes, kurioms paskaičiuoti naudotos rūšių jautrumo vertės iš 10–30 m gylio (kito nuo 3,2 iki 5,1; vidutiniškai  $4,2 \pm 0,5$ ) (11 pav.). Bentoso kokybės indekso vertės, kurioms paskaičiuoti naudotos jautrumo vertės iš mažesnių gylių, daugeliui mėginių buvo pervertintos. Skirtumas tarp bentoso kokybės indekso verčių, kurioms gauti buvo panaudotos jautrumo vertės iš dviejų gylio intervalų (10–30 m ir 30–50 m), mažėjo nuo 0,6 iki 0,1 indekso vertėms didėjant. Indekso vertės, kurioms naudotos rūšių jautrumo vertės iš 10–70 m gylio intervalo, buvo statistiškai reikšmingai mažesnės už reikšmes, kurioms apskaičiuoti naudotos jautrumo vertės iš 30–50 m gylio (kito nuo 3,3 iki 5,7) (11 pav.). Analizuojant šiuos gylio intervalus (10–70 m ir 30–50 m), skirtumas tarp indekso verčių augo didėjant indekso vertėms. Bentoso kokybės indekso vertės, kurioms paskaičiuoti buvo naudojamos jautrumo vertės iš 50–70 m gylio intervalo, kito nuo 2,3 iki 3,5 (vidutiniškai  $2,9 \pm 0,3$ ) ir buvo reikšmingai mažesnės, negu indekso vertės, kurioms paskaičiuoti buvo naudojamos rūšių jautrumo reikšmės iš 10–70 m gylio intervalo (11 pav.). Indekso vertės, kurioms apskaičiuoti panaudotos jautrumo vertės iš 50–70 m gylio, buvo pervertintos apie 2,5 karto neaptikus ryšio tarp bentoso kokybės indekso verčių, kurioms apskaičiuoti naudotos rūšių jautrumo vertės iš 10–70 m gylio intervalo.

**Antrame poskyryje** pateikiami bendrijų struktūros poveikio rezultatai bentoso kokybės indekso vertėms, kurioms paskaičiuoti buvo naudotos rūšių jautrumo ver-

tės, gautos iš skirtingų dominuojančių bendrijų – dvigeldžio moliusko *L. balthica* ir daugiašerės kirmėlės *Marenzelleria* spp. bei neišskyrus bendrijų (bendras duomenų rinkinys) (12 pav., 13 pav.). Bentoso kokybės indekso vertės, kurioms gauti buvo naudojamos rūšių jautrumo vertės iš bendro (neišskiriant bendrijų) duomenų rinkinio, kito nuo 1,6 iki 3,4 (vidutiniškai  $2,5 \pm 0,5$ ) ir buvo statistiškai reikšmingai mažesnės (Vilkoksono rangų ženklų testas,  $p < 0,01$ ,  $n=105$ ) negu indekso vertės, kurioms apskaičiuoti naudotos rūšių jautrumo vertės iš *L. balthica* bendrijos (vidutiniškai  $3,5 \pm 0,4$ ) (13 pav.). Bentoso kokybės indekso vertės, kurioms apskaičiuoti naudotos rūšių jautrumo vertės iš *Marenzelleria* spp. bendrijos (vidutiniškai  $1,5 \pm 0,4$ ) statistiškai reikšmingai (Mano-Vitnio testas,  $p < 0,01$ ,  $n=51$ ) skyrėsi ir buvo apie 2 kartus mažesnės negu vertės, apskaičiuotos naudojant rūšių jautrumo reikšmes iš *L. balthica* bendrijos (vidutiniškai  $3,5 \pm 0,4$ ) (12 pav. ir 13 pav.).

**Trečiame poskyryje** pateikiama bentoso kokybės indekso verčių poveikių analizė, kurioms apskaičiuoti buvo naudotos makrozoobentoso rūšių jautrumo vertės iš eutrofikacijos, grunto pylimo ir dugninio tralavimo poveikio vietų.

**Bentoso kokybės indekso verčių pokyčiai esant eutrofikacijos poveikiui.** Eutrofikacijos poveikis bentoso kokybės indekso vertėms buvo analizuotas naudojant rūšių jautrumo vertes, apskaičiuotas iš skirtingų duomenų rinkinių – foninių vietų ir poveikio bei foninių vietų (bendras duomenų rinkinys) (14 pav.). Bentoso kokybės indekso vertės buvo statistiškai reikšmingai (Vilkoksono rangų ženklų testas,  $p < 0,0001$ ,  $n=65$ ) mažesnės, kur jautrumo vertės apskaičiuotos naudojant bendrą duomenų rinkinį, už bentoso kokybės indekso reikšmes, kur jautrumo vertės apskaičiuotos tik iš foninių vietų duomenų rinkinio. Poveikio ir foninių vietų išskyrimas pagal bentoso kokybės indekso vertes buvo geresnis naudojant rūšių jautrumo vertes, apskaičiuotas iš bendrų vietų duomenų rinkinio, kur indekso verčių persidengimo intervalas buvo siauresnis ( $1,9-2,6$ ) palyginus su vertėmis, kurios apskaičiuotos naudojant rūšių jautrumo vertes, gautos iš foninių vietų duomenų rinkinio ( $3,1-4,1$ ). Žemiau bentoso kokybės indekso verčių  $1,9-3,2$  ribos (priklausomai nuo duomenų rinkinio, kuris naudotas rūšių jautrumo vertėms apskaičiuoti) buvo rasti tik poveikio mėginiai, tačiau tai sudarė tik 42 % visų poveikio mėginių, kurie pateko į šį intervalą. Skirtingai, didesnės indekso vertės už  $2,4-4,1$  ribą buvo apskaičiuotos tik foninių vietų mėginiais, tai sudarė 48 % visų foninių mėginių (14 pav.).

**Bentoso kokybės indekso verčių pokyčiai esant grunto pylimo poveikiui.** Grunto pylimo poveikis bentoso kokybės indekso vertėms buvo analizuotas naudojant rūšių jautrumo vertes, apskaičiuotas iš skirtingų duomenų rinkinių – foninių vietų ir poveikio bei foninių vietų (bendras duomenų rinkinys) (15 pav.). Bentoso kokybės indekso vertės buvo statistiškai reikšmingai (Vilkoksono rangų ženklų testas,  $p < 0,01$ ,  $n=118$ ) mažesnės, kur jautrumo vertės apskaičiuotos naudojant bendrą duomenų rinkinį, už indekso vertes, kur jautrumo vertės apskaičiuotos tik iš foninių vietų duomenų rinkinio. Poveikio vietose bentoso kokybės indekso vertės svyravo nuo 1,9

iki 3,8 (vidutiniškai  $3,0 \pm 0,5$ ) priklausomai nuo rūšių jautrumo verčių, apskaičiuotų naudojant tik foninių vietų duomenų rinkinį, ir nuo 1,7 iki 3,6 (vidutiniškai  $2,8 \pm 0,2$ ), kur rūšių jautrumo vertės apskaičiuotos naudojant poveikio ir foninių vietų duomenų rinkinį. Skirtingai, indekso vertės tik foninių vietų mėginiuose kito nuo 2,8 iki 3,9 ir nuo 2,7 iki 3,7 kai indekso vertėms gauti buvo naudotos rūšių jautrumo vertės iš poveikio ir foninių vietų mėginių. Žemiau bentoso kokybės indekso verčių 2,6–2,7 ribos (priklausomai nuo duomenų rinkinio, kuris naudotas rūšių jautrumo vertėms apskaičiuoti) buvo rasti tik poveikio vietų mėginiai, tačiau tai sudarė tik 27 % visų poveikio vietų mėginių, kurie pateko į šį intervalą. Skirtingai, indekso vertės tarp 3,6–3,7 ribos buvo apskaičiuotos tik foninių vietų mėginiams, o verčių riba tarp 2,6 ir 3,6 sudarė 90 % visų mėginių, iš kurių 43 % ir 80 % buvo identifikuoti kaip atitinkamai poveikio ir foninių vietų mėginiai (15 pav.).

#### **Bentoso kokybės indekso verčių pokyčiai esant dugninio tralavimo poveikiui.**

Duginio tralavimo poveikis bentoso kokybės indekso vertėms buvo analizuotas naudojant rūšių jautrumo vertes, apskaičiuotas iš skirtingų duomenų rinkinių – foninių vietų ir poveikio bei foninių vietų (bendras duomenų rinkinys) (16 pav.). Bentoso kokybės indekso vertės, kurios apskaičiuotos panaudojant rūšių jautrumo reikšmes iš foninių vietų mėginių, kito nuo 0,4 iki 2,4 (vidutiniškai  $1,3 \pm 0,7$ ) ir buvo statistiškai reikšmingai mažesnės (Vilkoksono rangų ženklų testas,  $p < 0,001$ ,  $n = 48$ ) palyginus su vertėmis (kito nuo 0,2 iki 1,6), kurioms gauti buvo naudojamos rūšių jautrumo vertės iš bendro poveikio ir foninių vietų duomenų rinkinio. Analizuojant tik poveikio vietų mėginius, bentoso kokybės indekso vertės, kurioms gauti buvo naudojamos rūšių jautrumo vertės iš poveikio ir foninių vietų, buvo statistiškai reikšmingai mažesnės (Vilkoksono rangų ženklų,  $p < 0,001$ ,  $n = 21$ ) už indekso reikšmes, kurios apskaičiuotos naudojant jautrumo vertes iš foninių vietų duomenų rinkinio. Skirtumas tarp bentoso kokybės indekso verčių, apskaičiuotų naudojant poveikio ir foninių bei tik foninių vietų duomenų rinkinius, didėjo augant indekso vertėms rodydamos didesnę riziką pervertinti indekso vertes, jei poveikio vietų mėginiai nedengtų poveikio gradiento ir rūšių jautrumo vertės būtų apskaičiuotos tik iš foninių vietų duomenų rinkinių. Priklausomai nuo analizuoto duomenų rinkinio, kurie buvo naudoti jautrumo vertėms apskaičiuoti, bentoso kokybės indekso vertės žemiau 0,5–0,8 rastos tik poveikio vietų mėginiuose, o didesnės vertės nei 1,3–1,6 – tik foninių vietų mėginiuose (16 pav.). Bentoso kokybės indekso verčių intervalai buvo panašūs naudojant poveikio ir foninių ir tik foninių vietų rūšių jautrumo vertes.

**Ketvirtame skyriuje**, kuris sudarytas iš trijų poskyrių, pristatomi bentoso kokybės indekso verčių validacijos rezultatai. Vertės buvo validuojamos panaudojant regresijos metodą su eutrofikacijos procesą apibūdinančiais parametrais ir su rūšių jautrumo grupėmis. Taip pat indekso vertės buvo testuojamos panaudojant Signalo aptikimo teorijos metodą su eutrofikacijos parametrais priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje bei nustatant geros–vidutinės vandens būklės slenksines vertes priekrantėje.



**Pirmame poskyryje** pateikiamos apskaičiuotos rūšių jautrumo vertės, gautos 11 makrozoobentos rūšių priekrantėje (iki 20 m gylio). Šios vertės kito nuo 2,3 iki 5,8 ir lygiais intervalais buvo suskirstytos į keturias grupes, pagal kurias buvo charakterizuojamas rūšių jautrumas. Šių rūšių jautrumo grupių santykinis gausumas keitėsi keičiantis bentos kokybės indekso vertėms (1,1–3,9), kurios buvo panaudotos vandens būklės klasėms ir slenkstinėms vertėms nustatyti (17 pav., 11 lent.).

**Antrame poskyryje** pateikiami bentos kokybės indekso verčių validacijos su Chl-*a*, bendro fosforo ir azoto koncentracijos rezultatai priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje. Kuršių marių vandenų sklaidos zonoje jūroje Chl-*a* koncentracijos buvo 6–7 kartus didesnės negu priekrantės mėginiuose ( $27,4 \pm 2,0$  mg/m<sup>3</sup> ir  $4,4 \pm 1,0$  mg/m<sup>3</sup> atitinkamai) (18 pav.), tačiau vertės kito panašiai priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje (variacijos koeficientas 0,05 ir 0,02 mg/m<sup>3</sup> atitinkamai) (18 pav.). Priekrantėje tarp indekso verčių ir Chl-*a* koncentracijos buvo rastas stiprus ir statistiškai reikšmingas ryšys ( $R=0,861$ ,  $p=0,0001$ ), kur Chl-*a* koncentracijos 1 mg/m<sup>3</sup> padidėjimas atitiko vidutinį indekso verčių 0,6 sumažėjimą. Skirtingai negu priekrantėje, Kuršių marių vandenų sklaidos zonoje jūroje nustatytas silpnas ryšys ( $R=0,396$ ,  $p=0,145$ ) tarp indekso verčių ir Chl-*a*, kur indekso vertės kito panašiai kaip ir Chl-*a* vertės (18 pav.). Indekso vertės buvo validuotos nustatant vandens būklės klases priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje, kur slenkstinės vertės buvo „atgal“ suskaičiuotos naudojant Chl-*a* koncentracijas, kurių slenkstinės vertės gerai–vidutinei vandens būklės klasei buvo nustatytos naudojant statistinius metodus bei ekspertinį vertinimą (slenkstinė Chl-*a* vertė gerai–vidutinei vandens būklės klasei priekrantės vandenims 4,8 mg/m<sup>3</sup> ir Kuršių marių vandenų sklaidos zonoje jūroje 25,7 mg/m<sup>3</sup> pagal Vandens politikos direktyvos Lietuvos paviršinio vandens vertinimo metodologiją; Langas ir kt. 2009). Tokiu būdu apskaičiuota bentos kokybės indekso geros–vidutinės vandens būklės klasės slenkstinė vertė sutapo su indekso slenkstine verte, kuri buvo apskaičiuota pagal makrozoobentos rūšių jautrumo grupių santykinio gausumo pokyčius (12 lent.).

Bentos kokybės indekso vertės kito nuo 1,7 iki 3,4 priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje validuojant jas su bendro fosforo ir azoto koncentracijomis. Vidutinės bendro azoto vertės buvo statistiškai reikšmingai didesnės Kuršių marių vandenų sklaidos zonoje jūroje, negu priekrantėje ( $51 \pm 17$  µg/l ir  $35 \pm 11$  µg/l atitinkamai,  $t=-3,783$ ,  $p=0,0006$ ) (19 pav.). Šie rezultatai paaiškina neigiamą druskingumo ir bendro azoto koncentracijos ryšį Kuršių marių vandenų sklaidos zonoje jūroje ( $r=-0,72$ ,  $p<0,001$ , druskingumo intervalas nuo 3,3 iki 7,1) ir nereikšmingą parametru ryšį priekrantėje ( $r=-0,02$ ,  $p<0,001$ , druskingumo intervalas nuo 6,3 iki 7,4 ‰). Bendro fosforo koncentracija buvo panaši Kuršių marių vandenų sklaidos zonoje jūroje ir priekrantėje ( $3,8 \pm 1,0$  µg/l ir  $3,4 \pm 1,0$  µg/l atitinkamai), kur nustatytas labai silpnas ryšys su druskingumo parametru (20 pav.).

**Trečiame poskyryje** bentoso kokybės indekso vertės buvo testuojamos naudojant Signalo aptikimo teorijos metodą priekrantėje ir Kuršių marių vandenų sklaidos zonoje jūroje atskirai (10–20 m gylyje). Naudojant AUC klasifikaciją pagal Hale ir Heltshe (2008) priimtinas bentoso kokybės indekso verčių atsakas ( $AUC \geq 0,70$ ) buvo rastas visiems analizuotiems eutrofikacijos procesą apibūdinantiems parametrams priekrantėje. Geriausias indekso atsakas buvo rastas į *Chl-a* ( $AUC=0,75$ ) ir bendrą fosforo ( $AUC=0,74$ ) koncentraciją (21 pav.). Kuršių marių vandenų sklaidos zonoje jūroje indekso atsakas į *Chl-a* ir bendrą fosforo koncentraciją ( $AUC=0,56$  abiemis parametrais) buvo prastas, tačiau į bendrą azoto koncentraciją indeksas sureagavo puikiai ( $AUC=0,87$ ) (21 pav.). Tiksliausia bentoso kokybės indekso slenkstinė vertė pagal indekso jautrumo ir specifiškumo sumą ir atsaką į *Chl-a* koncentraciją buvo 2,56 (indekso specifiškumas 0,75 ir jautrumas 0,86; 22 pav.). Geros–vidutinės būklės, nustatytos pagal *Chl-a* koncentracijos slenkstines vertes, paplitimas buvo 0,69 (16 iš 23 mėginių). „Griežta“ bentoso kokybės indekso slenkstinė vertė tarp geros ir vidutinės vandens klasės buvo 2,45, kai teigiamos numanomos reikšmės buvo didesnės (90 %), o neigiamos numanomos reikšmės mažesnės (55 %). „Lanksti“ bentoso kokybės indekso slenkstinė vertė buvo 3,05, kur palyginus teigiamos numanomos reikšmės buvo mažesnės, o neigiamos numanomos reikšmės didesnės (80 % ir 100 % atitinkamai, 22 pav.).

## DISKUSIJA

Disertacijos diskusija sudaryta iš penkių skyrių: 1) Makrozoobentoso rūšių jautrumo verčių ir bentoso kokybės indekso reikšmių pokyčiai keičiantis aplinkos gradientams, 2) Makrozoobentoso rūšių jautrumo reikšmių pokyčiai, priklausantys nuo poveikio mėginių duomenų rinkinių pilnumo, 3) Makrozoobentoso rūšių jautrumo reikšmių priklausomybė nuo tiriamo poveikio tipo, 4) Bentoso kokybės indekso validacija naudojant Signalo aptikimo teorijos metodą, 5) Vandens būklės pietrytinėje Baltijos jūros dalyje vertinimo naudojant bentoso kokybės indeksą rekomendacijos.

**Pirmame skyriuje** aptariama, kad Baltijos jūroje druskingumo gradientas yra laikomas didžiausias rūšių paplitimą reguliuojantis faktorius, kur rūšių skaičius mažėja nuo pietvakarių iki šiaurinės jūros dalies paveikdamas makrozoobentoso rūšių bendrųjų struktūrą ir biologinę įvairovę. Atliktų studijų rezultatai parodė, kad druskingumas turi poveikį bentoso kokybės indekso ir rūšių jautrumo vertėms (Zettler ir kt. 2007; Schiele ir kt. 2016), o regioniniame lygmenyje vandens gylis tampa labai svarbus faktorius, lemiantis makrozoobentoso struktūros pokyčius ir rūšių jautrumo verčių skirtumus. Šių studijų rezultatai parodė, kad 4 iš 16 makrozoobentoso rūšių jautrumo vertės nekito analizuotame gylio gradientu. Stabiliausios rūšių jautrumo vertės buvo apskaičiuotos gausiausios ir dažniausiai aptinkamos rūšims, tokioms kaip *Marenzelleria* spp., *P. elegans* ir moliuskui *L. balthica*. Taip pat tyrimo rezultatai parodė, kad rūšių jautrumo



vertės mažėjo didėjant gyliui (giliau negu 50 m), panašūs rezultatai buvo rasti Švedijos vandenynse (Leonardsson ir kt. 2016). Šio darbo rezultatų analizė parodė, kad 30–40 m gylis daugeliui rūšių yra kritinis, kur rūšių jautrumo vertės gali būti pervertintos. Tolerantiškų rūšių dominavimas gilesniuose gyliuose, maža biologinė įvairovė ir rūšių gausumas bei aplinkos heterogeniškumas lemia mažas rūšių ir bentoso kokybės indekso vertes didesniuose gyliuose. Dėl šios priežasties vandens būklės vertinimas turi būti atliktas priekrantėje ir atvirose vandenynse (haloklino zonoje) atskirai.

Be vandens druskingumo ir gylio, kiti svarbūs aplinkos parametrai, tokie kaip dugno vientisumas, maisto prieinamumas, deguonies koncentracija, srovės tėkmė, vandens temperatūra, drumstumas ir substratas, daro įtaką makrozoobentoso bendrijų struktūrai ir rūšių paplitimui (Bromley 1996; Olenin 1997; Pearson ir Rosenberg 1978; Bondorff 2006). Rūšių paplitimo ribos, taip pat rūšių genetinė įvairovė priekrantėje ir atviroje jūroje gali turėti įtakos rūšies jautrumo vertinimui. Kiti studijų rezultatai parodė, kad invazinių rūšių didelis gausumas vertinamoje bendrijoje gali turėti įtakos bendrijų struktūrai ir bentoso kokybės indekso verčių pokyčiams (Zaiko ir Daunys 2015).

Rūšių jautrumo ir bentoso kokybės indekso verčių pastovumo analizė priekrantėje parodė reikšmingus skirtumus tarp dviejų dominuojančių bendrijų *L. balthica* ir *Marenzelleria* spp. Rūšių įvairovė ir gausumas *Marenzelleria* spp. bendrijoje buvo mažesnis – tai lėmė beveik 50 % mažesnes rūšių jautrumo vertes palyginus su *L. balthica* bendrijoje rastomis apskaičiuotomis rūšių vertėmis. Šie skirtumai galėtų būti paaiškunami bendrijoms tinkamu gyventi nuosėdų sudėties duomenų analize, tačiau šios studijos metu dugno nuosėdų duomenys nebuvo analizuoti. Bentoso kokybės indekso vertės statistiškai reikšmingai skyrėsi, palyginus kai jos buvo apskaičiuotos abiejose analizuotose bendrijose ir neišskyrus bendrijų (bendras duomenų rinkinys). Vertinant vandens būklę svarbu analizuoti makrozoobentoso bendrijų gausumo pokyčius ir bentoso kokybės indekso atsaką į bendrijų struktūrą bei rūšinę įvairovę.

Rūšių jautrumo verčių ir indekso atsako į gylio gradiento ir aplinkos parametrų heterogeniškumą tame pačiame gylyje analizė parodė, kad ryšys tarp galimos antropogeninės veiklos ir rūšies būsenos gali būti netiesioginis, todėl visi anksčiau aptarti aspektai turėtų būti apsvarstyti vertinant rūšių jautrumo vertes ir indekso pokyčius.

**Antrame skyriuje** aptariama, kad rūšių jautrumo vertės gali būti priklausomos nuo biologinės įvairovės paplitimo, kuris padengtas duomenų rinkiniu, ribų. Mažas arba nepakankamas mėginių skaičius iš paveiktų vietų gali nepakankamai atspindėti rūšių gausumo dinamiką ir tai gali paveikti rūšių jautrumo grupių skirstymą bei galiausiai visą vandens būklės vertinimą. Leonardsson ir kt. (2016) siūlė vandens būklės vertinimui naudoti bent po 20 mėginių iš paveiktų ir nepaveiktų vietų norint turėti tinkamą poveikio gradiento padengimą analizuojant rūšių jautrumą ir bentoso kokybės indekso vertes. Šios studijos metu mėginių skaičius svyravo priklausomai nuo pasirinkto poveikio tipo, tačiau visada atitiko šias sąlygas.

Įprastai atsparios rūšies *L. balthica* jautrumas keičiant duomenų rinkinius buvo paveiktas labiau palyginus su santykinai jautria *P. elegans* rūšimi, kuri, yra žinoma, neigiamai reaguoja į grunto pylimo poveikį (Olenin 1992). Šie rezultatai rodo, kad rūšių, kurios stipriai reaguoja į poveikį ir jų gausumas sumažėja pagal antropogeninio poveikio gradientą, jautrumo vertės yra santykinai stabilios. Tokiais atvejais papildomas mėginių skaičius iš paveiktų vietų (mažas ES50), kur rūšių gausumas bus arba labai mažas, arba nulinis, turės mažą poveikį rūšių jautrumo vėrtėms. Skirtingai, tolerantiškos rūšys yra gausios poveikio vietose, taip pat randamos viduriniame poveikio lygmenyje, taigi papildomi duomenys iš tokių vietų turėtų reikšmingą įtaką tokių rūšių jautrumo vėrtėms (23 pav.).

Vertinant rūšių jautrumo vėrcių įtaką vandens būklės vertinimui, bentoso kokybės indekso vėrtės gali būti 60–100 % aukštesnės, kurioms apskaičiuoti naudotos rūšių jautrumo vėrtės, apskaičiuotos iš limituoto poveikio vietų mėginių skaičiaus. Vertinant rūšių jautrumus ir grupes, svarbu turėti patikimus duomenis, kurie padengtų rūšių atsaką į analizuojamus poveikius. Neskaitant to, kad rūšių jautrumo vėrtės turėjo didelę įtaką bentoso kokybės indekso vėrtėms, rūšių jautrumo vėrcių, gautų iš skirtingai apibūdintų paveiktų ir nepaveiktų poveikio vietų, poveikis buvo nereikšmingas. Tai parodė, kad reikšmingi bentoso kokybės indekso pokyčiai, tikėtina, yra sisteminio pobūdžio sutinkant su Leonardsson ir kt. (2016) pasiūlytu būdu naudoti regresijos modelį vertinant gylio poveikį ir taip panaikinant erdvinę variaciją.

**Trečiame skyriuje** aptariama, kad Bentoso kokybės indeksas reaguoja į organinės apkrovos, hipoksijos, sunkiųjų metalų, nuotekų, fizinių trikdžių ir dugno tralavimo poveikius (Josefson ir kt. 2009; Gislason ir kt. 2017), kur rūšių jautrumo vėrtės gali priklausyti nuo analizuojamo poveikių tipo. Šio tyrimo metu buvo analizuoti trys poveikio tipai (eutrofikacijos proceso, grunto pylimo ir dugninio tralavimo), kur poveikio vietos išsidėsčiusios 40–50 km atstumu. Eutrofikacija yra erdvinis poveikis, paplitęs didesniuose plotuose ir laike, palyginus su taškiniais, ir santykinai trumpu poveikiu pasižymintys poveikiai, kaip grunto pylimas ar dugno tralavimas. Duomenų analizės metu pridėjus poveikio mėginius iš visų tipų poveikio rūšių jautrumo vėrtės buvo modifikuotos. Eutrofikacijos rajone rastų rūšių jautrumas buvo aukščiausias, palyginus su grunto pylimo ir dugno tralavimo poveikių rūšių jautrumu. Pavyzdžiui, tarp 40 iki 65 metrų gylio daugiašerė kirmėlė *B. sarsi* buvo viena iš jautriausių rūšių grunto pylimo rajone ir tuo pačiu metu turėjo mažiausią jautrumo vėrtę dugno tralavimo zonoje. Toks pat rūšių jautrumo pokytis buvo rastas ir kitai daugiašerei kirmėlei *Marenzelleria* spp., tačiau šios tendencijos buvo nustatytos tik foninėse vietose, neat-sižvelgiant į rūšių jautrumo atsaką į analizuojamus poveikius.

Kiti veiksniai, tokie kaip rūšies judrumas ar pelaginė vystymosi stadija, gali reikšmingai sumažinti rūšių jautrumo vėrtes dėl didelių rūšių sugebėjimų atsikurti. Tokiais atvejais rūšis, būdama jautri poveikiams, gali būti viena iš pirmųjų kolonizatorių poveikio vietų mėginiuose dėl jų ankstyvos atsikūrimo stadijos, kas ypatingai svarbu taškinio poveikio tipams, tokiems kaip grunto pylimas ar dugninis tralavimas. Šio tyrimo metu

hipotezę patvirtino rezultatai apie daugiašerę kirmėlę *B. sarsi*, kuri dugninio tralavimo vietose buvo tolerantiška, nes yra greitai įsikurianti rūšis periodiškai atsinaujinančiose vietose, kuriose dažniausiai yra maža deguonies koncentracija. Taip pat ši tendencija rasta ir priapulidų rūšiai *H. spinulosus*, kuri pasižymi aukštu atsparumu anoksijai (Janssen ir Oeschger 1992; Oeschger ir Vetter 1992). Nors iš dalies rūšių jautrumo vertės keitėsi dėl analizuojamų poveikių tipo, akivaizdu, kad kintančios aplinkos sąlygos (ypač gylis ir bendrųjų struktūros pokyčiai) turėjo didžiausią įtaką rūšių jautrumo pokyčiams.

**Ketvirtame skyriuje** aptariama, kad Baltijos jūroje eutrofikacija yra vienas iš svarbiausių poveikių, turinčių įtakos skirtingoms ekosistemos sudedamosioms dalims – nuo fitoplanktono iki makrozoobentos bendrųjų (HELCOM, 2009). Naudojant tradicinius statistikos metodus (tiesinę regresiją) nebuvo aptiktas bentoso kokybės indekso verčių atsakas į tiriamus eutrofikacijos parametrus (išskyrus Chl-*a* koncentraciją). Pritaikius Signalo aptikimo teorijos metodą buvo rasti reikšmingi ryšiai tarp bentoso kokybės indekso ir analizuotų eutrofikaciją apibūdinančių parametru prie krantėje, tačiau Kuršių marių vandenų sklaidos zonoje jūroje buvo rasti nepatikimi ryšiai tarp indekso ir Chl-*a* bei bendro fosforo koncentracijos.

Signalo aptikimo teorijos metodo taikymas gali padėti įvertinti aplinkos parametru pokyčius tam tikromis sąlygomis, nustatant indekso slenkstines vertes ir vertinant vandens kokybės būklę mokslškai pagrįstu būdu. Naudojantis šiuo metodu lengviau galima nuspręsti, ar indeksas reprezentatyvus ir tinkamas poveikiui aptikti. Atsižvelgiant į nustatytus tikslus, Signalo aptikimo teorijos metodas gali būti naudojamas rengiant stebėsenos programą ir atsakant į praktinius ekologinius ir valdymo klausimus, pvz., kaip tankiai rinkti makrozoobentos mėginius, norint nustatyti poveikį ir įvertinti aplinkos būklę, atsižvelgiant į konkrečias sąlygas, galimus triukšmo veiksnus. Labiausiai tiksli indekso slenkstinė vertė, kurią siūlo Signalo aptikimo teorijos metodas, ne visada gali būti geriausias pasirinkimas, nes kai kuriais atvejais tiriamoje teritorijoje, kai degradacija yra mažiau išreikšta, natūralus atsikūrimas vis dar yra įmanomas. Vertinant saugomų teritorijų plotus rekomenduojama pasirinkti maksimalias neigiamas numanomas reikšmes ir nustatyti „lanksčią“ indekso slenkstinę ribą. Jei bentoso kokybės indekso vertės vertinamos stipriai paveiktoje teritorijoje, rekomenduojama naudoti maksimalias teigiamas numanomas reikšmes ir griežtesnę indekso slenkstinę ribą.

**Penktame skyriuje** vertinant vandens būklę pietrytinėje Baltijos jūros dalyje naudojant bentoso kokybės indekso vertes pateikiamos detalios rekomendacijos:

1. Bentoso kokybės indekso vertės, naudojamos vandens būklei vertinti, turi būti naudojamos išskirtoms bendrijoms bei gylis ir druskingumo zonoms. Visi su vertinimu susiję aplinkos parametru duomenys turi būti kokybiškai apibūdinti ir charakterizuoti:

- 1.1. rūšys, identifikuotos 8 ar mažiau mėginiuose, turėtų būti išimtos iš analizuojamo duomenų rinkinio. Analizei naudojamos makrozoobentos rūšys būdingos tik minkštam dugniui;

1.2. duomenų rinkinyje, kur mėginiai rinkti iš didesnio gylio negu 40 m, tipiškos sėkliamėgės rūšys (tokios kaip *C. glaucum*, *S. shrubsolii*, *B. pilosa*, *M. arenaria*, *C. volutator*, *H. diversicolor*, *Hydrobia*) neturėtų būti įtrauktos į vertinimą;

1.3. vertinant priekrantės vandens būklę sekliu negu 40 m gylio, giliavandenės rūšys (tokios kaip *B. sarsi*, *H. spinulosus*, *S. entomon*, *M. affinis*, *Ostracoda* undet.) turėtų būti išimtos iš analizei naudojamo duomenų rinkinio;

1.4. dominuojančios makrozoobentos bendrijos, naudojamos vandens būklei vertinti, turėtų būti išskirtos pagal rūšių gausumą, biomasę, dugno nuosėdas ir kitus parametrus.

2. Naudojant makrozoobentos rūšių jautrumo vertes vandens būklei vertinti rekomenduojama naudoti atrinktus antropogeninės veiklos tipus, kurie turėtų būti mokslškai įvertinti, interpretuojant rezultatus:

2.1. norint aptikti tikslias indekso naudojimo ribas, rūšių jautrumo vertėms apskaičiuoti turėtų būti naudojami duomenų rinkiniai, sudaryti iš mažiausiai 20 mėginių, rinktų foninėse ir poveikio vietose;

2.2. norint sužinoti rūšių atsaką į poveikį, rūšių jautrumo vertės galėtų būti skaičiuojamos mėginiuose, kuriuose aptinkama 50 ir mažiau individų. Tokie mėginiai turėtų būti validuojami su apskaičiuota *ES50* verte ir rūšių skaičiumi analizuojamame mėginyje, tačiau apskaičiuota *ES50* vertė galėtų būti naudojama rūšių jautrumo vertėms apskaičiuoti, norint prailginti poveikio gradiento padengimą duomenų rinkinyje;

2.3. eutrofikacijos poveikio rajone mėginiai gali būti charakterizuojami naudojant *Chl-a* satelitinius duomenis (padidėjusi *Chl-a* koncentracija, vieno metų vėlavimas taikytas indekso vertėms); grunto pylimo rajone – poveikio vietoms išskirti naudojamos nustatytos grunto pylimo vietos, daugiašerės kirmėlės *P. elegans* ir kiautavėžio *Ostracoda* undet. gausumo pokyčiai, dugno nuosėdų duomenys, dugno tralavimo zonoje – akustinė informacija apie tralavimo žymių vietas, taip pat bendrųjų struktūrą.

3. Keturios rūšių jautrumo grupės, naudojamos bentos kokybės indekso atsakui vertinti, gali būti sudarytos jautrumo intervalą skirstant į lygias dalis.

3.1. Rūšių jautrumo grupių charakteristikos gali būti naudingos interpretuojant vandens kokybės klasių ribas ir atliekant vandens būklės vertinimą, tačiau šios vertės turi būti statistiškai patikrintos.

4. Vienų metų vėlavimas yra statistiškai reikšmingas tiriant bentos kokybės indekso ir pelaginių parametrų ryšį, o apytiksliai 3x3 km<sup>2</sup> atstumu eutrofikacijos procesą apibūdinančių parametrų mėginių rinkimo rajonas statistiškai reikšmingai paaiškina bentos kokybės indekso verčių pokyčius.

4.1. Signalo aptikimo teorijos metodas siūlomas bentos kokybės indekso validacijai, taip pat vandens būklės klasėms nustatyti pasirenkant tarp „lanksčių“ (maksimalios neigiamos numanomos reikšmės ir indekso jautrumas) ir „griežtų“ slenksčių (maksimalios teigiamos numanomos reikšmės ir indekso specifiškumas) verčių.

## IŠVADOS

1. Minkšto dugno makrozoobentos rūšių jautrumo vertės yra priklausomos nuo gylio. Gyliuose tarp 10–70 m rūšių jautrumo vertės buvo stabilios 4 plačiai paplitusioms rūšims (*Marenzelleria* spp., *P. elegans*, *L. balthica*, *Oligochaeta* undet.) iš 16 analizuotų. 30–40 m gylys apibūdintas kaip kritinis gylis, kurį naudojant galimas tiek sėkliaamėgių (pvz., *C. glaucum*), tiek giliavandenių rūšių (pvz., *S. entomon*) jautrumo verčių perversinimas. Visų tirtų rūšių jautrumo vertės mažėjo didėjant gyliui (nuo 50 m).
2. Rūšių jautrumo ir bentoso kokybės indekso verčių pastovumo analizė parodė reikšmingus skirtumus tarp dviejų dominuojančių bendrijų *L. balthica* ir *Marenzelleria* spp. tame pačiame gylyje. Rūšių įvairovė ir gausumas *Marenzelleria* spp. bendrijoje buvo mažesnis, tai lėmė iki dviejų kartų mažesnės rūšių jautrumo ir bentoso kokybės indekso vertės.
3. Tolerantiškų rūšių jautrumo vertės yra priklausomos nuo poveikio vietų padengimo, o jautrios rūšys į šį efektą nereaguoja.
4. Tolerantiškos ir jautrios rūšys skirtingai reaguoja į antropogeninės veiklos poveikius vertinant aplinkos būklę. Į eutrofikacijos poveikį geriausiai reaguoja moliuskų rūšys kaip *M. arenaria*, *C. glaucum*, *Hydrobia* sp. ir šoniplauka *B. pilosa*, kur grunto pylimo ir dugno tralavimo poveikį atspindi *P. elegans* – kiautavėžių ir *Marenzelleria* spp. – kiautavėžių atitinkamai. Rūšių tolerantiškumas į tirtus antropogeninius poveikius buvo skirtingas, tačiau moliusko *L. balthica* rūšis buvo tolerantiškiausia visiems tirtiems poveikiams.
5. Signalo aptikimo teorijos metodas puikiai tinkamas indekso validacijai ir vandens būklės klasėms nustatyti. Skirtingai negu bentoso kokybės indekso validacija regresijos metodu, Signalo aptikimo teorijos metodas parodė priimtina indekso atsaką į bendrą azoto, fosforo ir Chl-*a* koncentraciją priekrantėje.



Klaipėdos universiteto leidykla

Romualda Chuševė

EVALUATION OF MACROZOOBENTHOS SPECIES SENSITIVITY AND APPLICATION  
OF BENTHIC QUALITY INDEX FOR THE SEABED STATUS ASSESSMENT  
OF THE SOUTH-EASTERN BALTIC SEA

*Doctoral dissertation*

MAKROZOOBENTOSO RŪŠIŲ JAUTRUMO VERTINIMAS IR BENTOSO KOKYBĖS  
INDEKSO TAIKYMAS VERTINANT PIETRYTINĖS BALTIJOS JŪROS DUGNO  
EKOSISTEMŲ BŪKLĘ

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