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Benthic Opportunistic Polychaete/Amphipod Ratio: An Indicator of Pollution or Modification of the Environment by Macroinvertebrates?

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Abstract: The development of sensitive indicators reflecting the state of the environment is an important issue for the monitoring of marine ecosystems. The spionid polychaete *Marenzelleria arctica* and pontoporeiid amphipod *Monoporeia affinis* are common macrobenthic species in the brackish Gulf of Finland (the easternmost Baltic Sea). This paper aims to apply the Benthic Opportunistic Polychaetes Amphipods (BOPA) and Benthic Opportunistic Annelid Amphipods (BO2A) indices based on the polychaete/amphipod ratio as indicators of the environmental state in this region. We analyzed the relationships between environmental variables and benthic indices based on samples from two benthic surveys in 2019 (10 sites) and 2020 (9 sites). The coastal sites were characterized by worse water quality (i.e., the higher concentration of hydrocarbons, total phosphorus and chlorophyll-a), but cleaner sediments (i.e., total phosphorus, organic carbon, polyaromatic hydrocarbons and trace metals) than offshore sites. The BOPA and BO2A correlated positively with the level of water pollution and negatively with sediment pollution. The activity of the benthic organisms seems to strongly influence the concentration of contaminants in sediments, so this factor hinders the use of BOPA and BO2A indices for the assessment of bottom sediments quality in the eastern Baltic Sea. At the same time, this study shows that BOPA and BO2A indices can be used in assessing water quality.



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Keywords: bioindicator; macrozoobenthos; environmental assessment; bioturbation; Baltic Sea; *Monoporeia affinis*; *Marenzelleria*

1. Introduction

The Baltic Sea is a sensitive environment affected by chemical pollution derived from multiple sources, such as atmospheric deposition, river discharges, and direct point sources [1]. According to the integrated assessment of the contamination status in the Baltic Sea, conducted by the Baltic Marine Environment Protection Commission, also known as the Helsinki Commission (HELCOM), for the period 2011–2016, all Baltic Sea basins failed to reach good environmental status [2]. The eastern Gulf of Finland (including the estuary of the Neva River) is located in the northeastern part of the Baltic Sea. It plays a crucial role in the formation of bioresources and the quality of the natural environment of the entire sea basin.

Seabed sediments are an important reservoir of harmful substances, so the organisms inhabiting them can play a critical role as sensitive indicators for monitoring pollutants in the aquatic environment. Macrozoobenthos are widely recognized as an indicator of change in environmental conditions in marine monitoring programs because benthic macroinvertebrates are relatively sedentary and long-lived. These features make benthic macrofauna ideal for impact assessment [3].

Various benthic indexes have been developed for assessments of the state of the surface waters under the EU Water Framework Directive [4,5]. These indexes are based on proportions of sensitive and tolerant taxa and (or) species richness of benthic communities. The applicability of the benthic indices in low-diversity communities of brackish water

areas such as estuaries and the Baltic Sea is questionable, since the effect of anthropogenic disturbance often cannot be entirely disentangled from the natural stress effects [6–8]. The main problem is that estuaries are inhabited by eurybiontic macroinvertebrates adapted to both natural (salinity fluctuations) and human-induced stress [7]. Moreover, species tolerances and sensitivities may change along salinity gradients [9,10]

The low diversity also results in a low number or complete absence of indicator species, which poses a restriction to effectively using many indices. For example, the robustness of the popular AMBI index is reduced when the number of taxa is lesser than three [11]. This problem especially concerns the Baltic Sea, where the number of macrobenthic species declines drastically from southwest to northeast [12,13]. The extreme poverty of the fauna in the easternmost and northernmost parts of the Baltic Sea leads to the formation of uniquely simple benthic communities of only a few species. Historically, in the eastern Gulf of Finland (Baltic Sea), the soft-bottom macrozoobenthos is dominated by two pontoporeiid amphipods, *Monoporeia affinis* and *Pontoporeia femorata*. By the 2000s, this species-poor community was enriched by the non-indigenous spionid polychaete *Marenzelleria arctica* and tubificid oligochaete *Tubificoides pseudogaster*, which became the dominant component of the macrozoobenthos [14,15].

Tubificid oligochaetes and spionid polychaetes are among the organisms most tolerant to stress associated with low oxygen and organic pollution [16–19]. On the other hand, pontoporeiid amphipods are known to be susceptible to different environmental disturbances such as eutrophication, pollution, and acidification [3,20,21]. In this regard, two new indicators based on the ratio of benthic opportunistic polychaetes and annelids (oligochaetes and polychaetes together) to amphipods proposed for estuarine and coastal communities are of interest [6,22,23].

Another aspect of annelids invasion is their potential role in reworking and bioturbation of marine sediments and burial of sedimented particulate matter. It is known that *Marenzelleria* spp. dig the bottom deeper than native Baltic species and essentially affect the exchange processes at the water–bottom interface, controlling the nutrient cycling [24,25] and potentially the release of contaminants sequestered in the sediment [26,27].

Although Benthic Opportunistic Polychaetes Amphipods (BOPA) and Benthic Opportunistic Annelids Amphipods (BO2A) indices are recognized as effective benthic indicators for assessing the ecological quality state of coastal water masses in many geographical localities [23], they were not yet applied to assess the state of the environment in the Baltic Sea. The aim of this study is to test the ratio of benthic opportunistic annelids to pontoporeiid amphipod as an indicator of the environmental state in the Gulf of Finland and assess the possibility for the application of BOPA and BO2A indices to the brackish water of the Baltic Sea. We analyzed the relationships between these indices and environmental variables to test their responses to water and sediment pollution.

2. Materials and Methods

2.1. Study Sites

The macrozoobenthos and bottom sediments were collected during research cruises on vessel “Maria” in August 2019 and vessel “SN-1303” in September 2020 at 15 sites, of which four sites were sampled twice in 2019 and 2020, in the eastern Gulf of Finland at depths from 27 to 52 m (Figure 1). The general hydrography and environmental state of this area were described in [28]. The deep-water temperature is low (usually 2–5 °C) throughout most of the year. Near-bottom salinity varies between 5 and 7 PSU. The substantial oxygen depletion (up to the formation of hypoxic conditions in some years) occurs in late summer–early autumn [29,30]. The bottom sediments at the study sites in the Gulf of Finland are soft gray and brown silt.

The eastern Gulf of Finland is among the most polluted regions in the Baltic Sea [2,28]. This semi-enclosed region drains the extensive economically developed catchment area, including St. Petersburg and its surroundings, with about seven million people. Shipping

is another source of pollution. The Gulf of Finland is one of the areas in the world with the most intense maritime traffic.

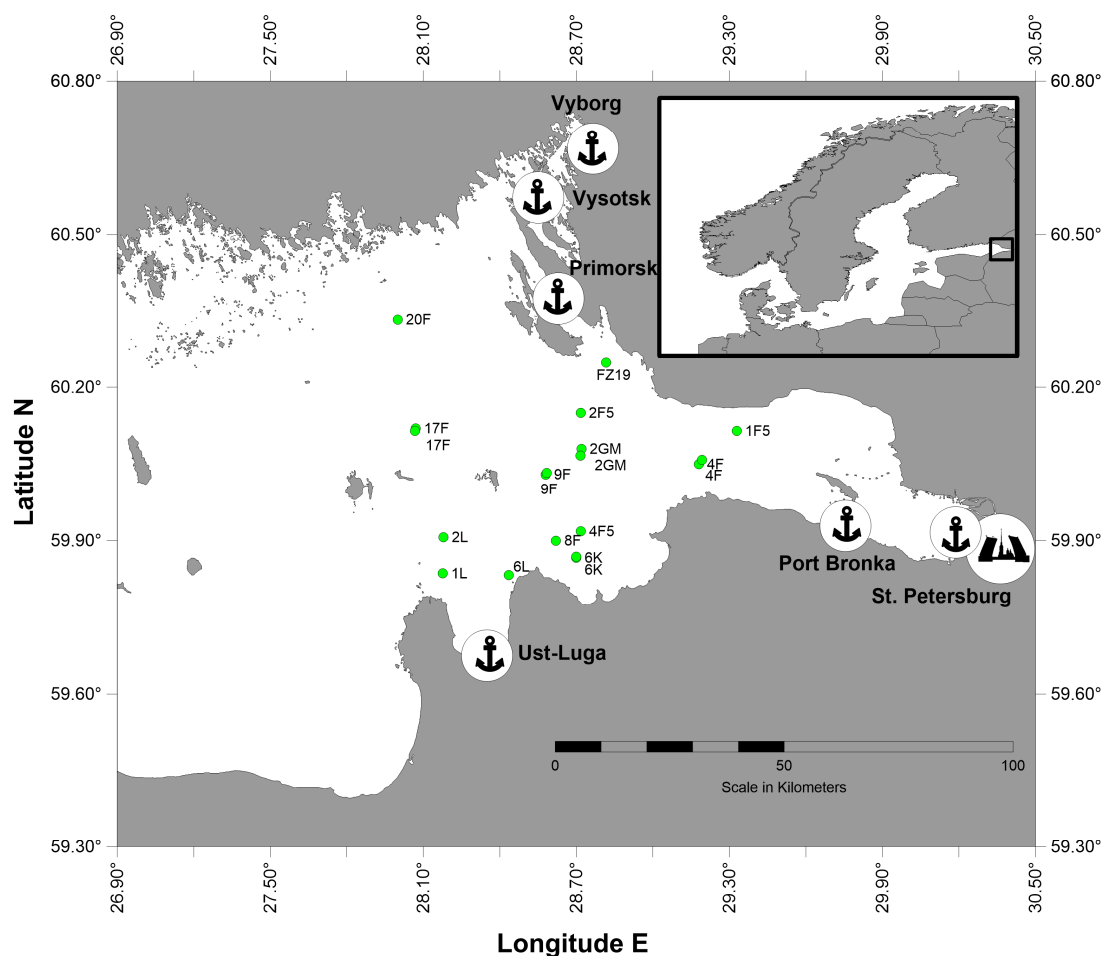


Figure 1. Sampling sites in the eastern Gulf of Finland.

2.2. Sampling Method and Analyses

Macrozoobenthos was collected by Van-Veen grab (sampling area: 0.025 m²). At each site, three samples were taken. The samples were sieved through screens with mesh sizes of 0.4 mm and fixed in a 4% formalin solution. The species composition, abundance, and biomass (formalin-wet weight) were determined in the laboratory. We used the same grab to collect 1 kg of the bottom sediments for chemical analysis. The surface sediment layer (up to 3 cm) was taken for chemical analysis from all sampling sites. Temperature, salinity (CTD), and oxygen level (Winkler titration method) in the surface and the near-bottom waters were measured together with benthic and sediment sampling.

To estimate concentrations of total phosphorus (TP), total petroleum hydrocarbons (TH) and chlorophyll-a in water, the integral water samples were taken from the euphotic zone (from the surface to three Secchi depth). Phytoplankton was concentrated by filtering about 1 l water through membrane filters with a pore size of ~1 µm. Then, chlorophyll-a was measured in an acetone extraction using standard techniques [31]. TP was determined by the photometric method. TH was determined by UV fluorescence spectroscopy using a FLUORAT-02-3M spectrofluorometer (Nordinkraft-Sensor, Cherepovets, Russia). The hydrocarbons were extracted with carbon tetrachloride.

As recommended by core indicators' third holistic assessment of HELCOM (HOLAS III), we estimated concentrations in sediments of polycyclic aromatic hydrocarbons (PAH) and heavy metals: mercury (Hg), cadmium (Cd), and lead (Pb). In addition, zinc (Zn) and copper (Cu) were measured at the study sites, as their concentrations in sediments

often exceed threshold values. These hazardous substances from bottom sediment samples were detected in the accredited analytical laboratory LABCLUSTER in Russia (<https://labcluster.ru/> (accessed on 20 December 2022)). PAHs were analyzed by high-performance liquid chromatography (HPLC) on a fluorescent detector (method FR.1.31.2004.01279). The analysis of PAH compounds in bottom sediments mainly included extraction with organic solvents, purification and separation by HPLC with ultraviolet light. The metal content in bottom sediments was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) on an Agilent 7700x mass spectrometer (Japan). The standards used corresponded to the Russian state standard (GOST 8.315-2019 Reference materials of composition and properties of substances and materials). In samples of bottom sediments, the content of organic carbon (C) was determined by the Tyurin method, as a percentage of the dry weight of bottom sediments. More information on the analytical methods used is available in [32].

2.3. Indices

Each environmental variable was standardized as $X = (x - M)/SD$, where $(x - M)$ is the difference between each value and the overall mean for the data series, and SD is the standard deviation. Then, standardized values were averaged for water and sediments to obtain summarized indices of water (WP) and sediment pollution (SP), respectively.

Benthic Opportunistic Polychaetes Amphipods index (BOPA) can be expressed mathematically as [22]:

$$BOPA\ index = \log_{10} \left(\frac{f_p}{f_a + 1} + 1 \right)$$

where f_p is the opportunistic polychaete frequency, i.e., ratio of the number of opportunistic polychaetes to the total number of individuals in the sample; f_a is the amphipod frequency, i.e., ratio of the number of amphipods to the total number of individuals in the sample). The two '+1' terms in the equation are needed if f_p or f_a is null to allow the division operation and logarithmic transformation.

After the initial BOPA proposition, Dauvin and Ruellet [6] proposed adding the Clitellata (i.e., Hirudinea and Oligochaeta) to the opportunistic polychaeta in order to calculate the 'Benthic Opportunistic Annelida Amphipods' index (BO2A). The both indices are based on the principle of antagonism between sensitive species (amphipods) and opportunistic species (polychaetes and oligochaetes) [23]. Values of the BOPA and BO2A vary in the interval from 0 to lg 2.

We modified the BOPA index using \log_2 so that the values of the index vary between 0 and 1. In this form, the BOPA index is written:

$$BOPA\ index = \log_2 \left(\frac{f_p}{f_a + 1} + 1 \right)$$

where f_p is the opportunistic polychaete frequency, i.e., ratio of the number of spionid polychaetes to the total number of individuals in the sample; f_a is the amphipod frequency, i.e., ratio of the number of pontoporeiid amphipods to the total number of individuals in the sample).

Similarly, BO2A index was calculated according to the formula:

$$BO2A\ index = \log_2 \left(\frac{f_{p+o}}{f_a + 1} + 1 \right)$$

where f_{p+o} is the opportunistic annelids frequency, i.e., ratio of the sum number of spionid polychaetes and oligochaetes to the total number of individuals in the sample; f_a is the amphipod frequency, i.e., ratio of the number of pontoporeiid amphipods to the total number of individuals in the sample.

The BOPA and BO2A indices approach zero when opportunistic polychaetes (or annelids, i.e., sum of numbers of polychaetes and oligochaetes) were absent, testifying a

good environmental state. Both indices increase to value 1 when only polychaetes (annelids) form macrofauna, testifying a poor environmental state.

2.4. Statistics

The software package STATISTICA (version 12) was used for statistical analyses of the dataset. The Spearman rank correlation was calculated to find relationships between studied environmental variables and benthic indices. PCA (principal component analysis) was applied to visualize data and extract significant information from the whole dataset in a multidimensional space. Before analyses, all variables were standardized as $X = (x - M)/SD$, where $(x - M)$ is the difference between each value and the overall mean for the data series, and SD is the standard deviation.

3. Results

3.1. Environmental Variables

The studied periods in 2019 and 2020 differed greatly in the hydrographic conditions. In August 2019, the water column was characterized by a stable vertical stratification. There was a steep pycnocline at the depth of about 20 m. The near-bottom temperature was about 4 °C, and the salinity varied from 5.3 at the 30 m isobath to 7.3 psu at the depth of 52 m (Site 17F). By September 2020, the stratification was broken, resulting in warming of the near-bottom waters. The temperature increased up to 11–16 °C, and the salinity varied between 2.3 and 4.0 psu. Hypoxia during the study period was not recorded (Table S1). In August 2019, the near bottom oxygen level varied from 2.16 (Site 17F, depth 52 m) to 4.51 mL L⁻¹ (Site 8F, depth, 30 m). In September 2020, the oxygen situation improved (4.8–7.2 mL O₂ L⁻¹) because of autumn mixing.

Standardized values of pollution-related environmental variables used in the study are presented in Table S2. The concentration of all contaminants varied in wide limits. However, generally, coastal sites (especially near the northern shore) were characterized by worse (positive anomalies of WP index) water quality (Figure 2). In contrast, the more polluted sediments (positive anomalies of SP index) were more common for offshore sites (Figure 3).

3.2. Macrozoobenthos

In terms of abundance, macrozoobenthos was practically comprised of three taxa: polychaete *Marenzelleria* spp. (mainly *M. arctia*), amphipod *M. affinis* and oligochaetes. The large isopod *Saduria entomon* and bivalve *Macoma balthica* were commonly characterized by high biomass, but numerically their shares in total macrozoobenthos, as well as the share of chironomids, were negligible (Table 1). Oligochaetes were strongly dominated by two tubificid species; *Tubificoides pseudogaster* and *Potamothrix hammoniensis* contributed 68 and 22%, respectively, to the total abundance of oligochaetes (Figure S1).

The observed values of BOPA (from 0.10 to 0.94) covered almost the whole theoretically possible variation range of this index. The lowest BOPA values (good environmental state) were recorded at offshore sites (Figure 4).

BO2A index basically demonstrated the similar pattern of spatial distribution (Figure 5). Both indices were significantly correlated with each other with a Spearman correlation coefficient of 0.709 ($p = 0.0007$).

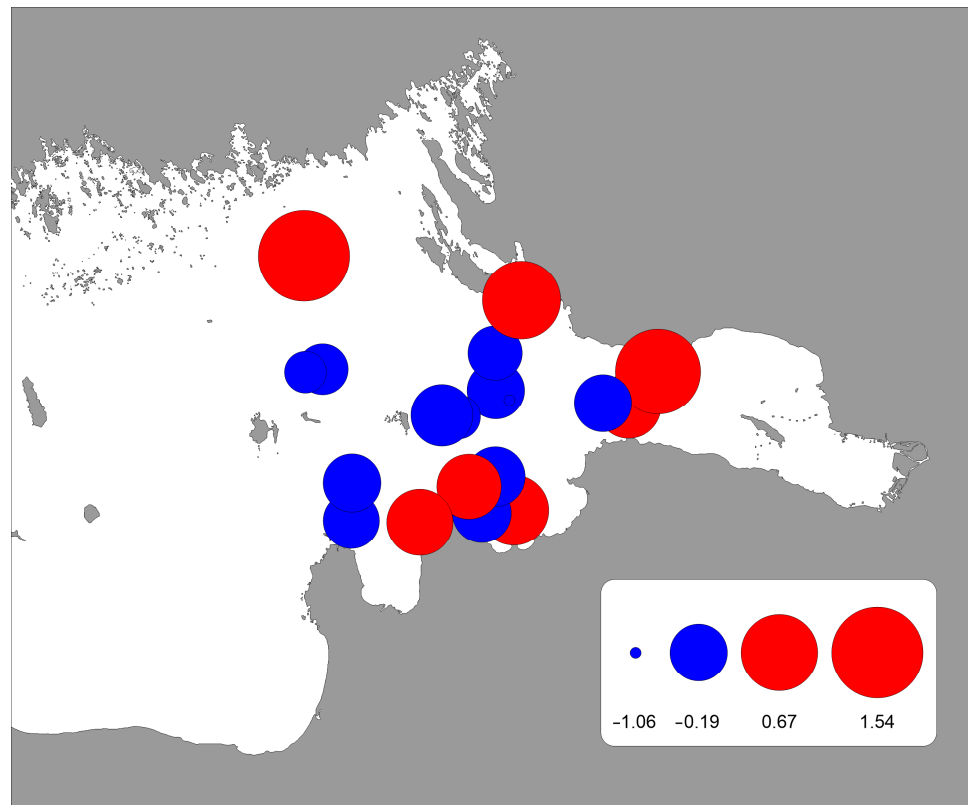


Figure 2. Water Pollution Index (WP) at sampling sites in the eastern Gulf of Finland. Blue and red cycles denote, respectively, negative and positive anomalies of this index.

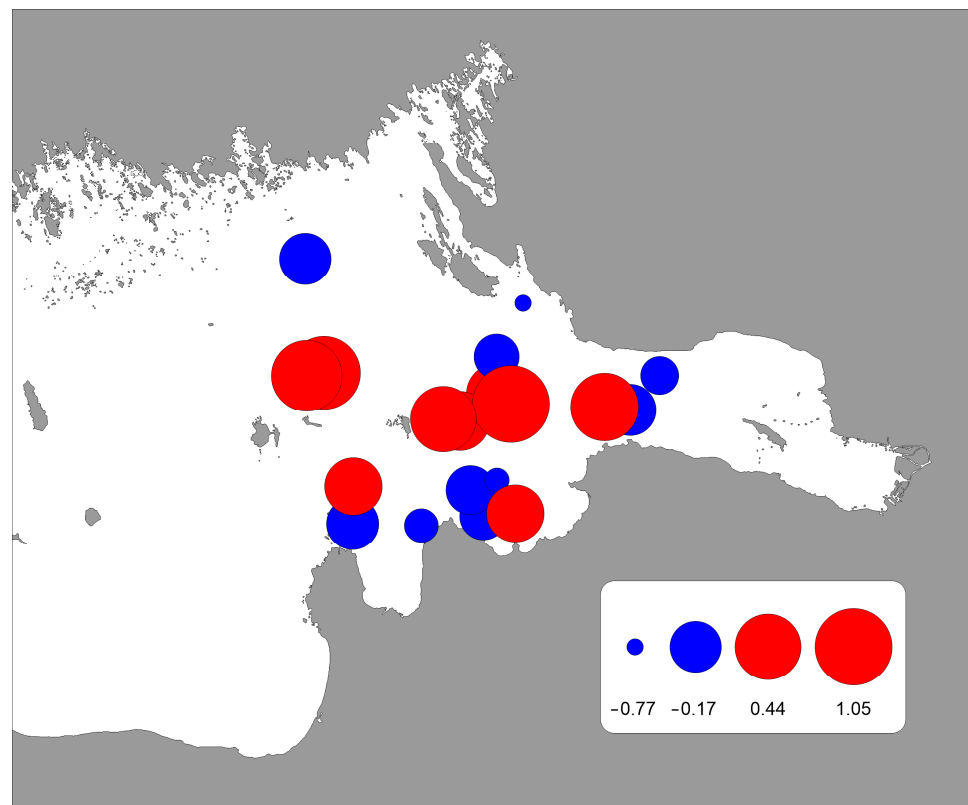


Figure 3. Sediment Pollution Index (SP) at sampling sites in the eastern Gulf of Finland. Blue and red cycles denote, respectively, negative and positive anomalies of this index.

Table 1. Abundance (ind. m⁻²) of macrobenthic taxa, Benthic Opportunistic Polychaetes Amphipods (BOPA) and Benthic Opportunistic Annelida Amphipods (BO2A) indices. Coastal sites are marked in bold.

Site	<i>Marenzelleria</i>	Oligochaeta	<i>M. affinis</i>	<i>S. entomon</i>	<i>M. balthica</i>	Chironomidae	Macrobenthos	BOPA	BO2A
2019									
4F	3280	160	60	-	20	-	3520	0.938	0.972
2GM	2800	7093	4253	-	13	-	14,160	0.204	0.620
17F	2020	720	400	-	100	-	3240	0.637	0.810
2F5	1190	4960	960	-	-	-	7110	0.198	0.817
9F	960	6440	1330	10	10	-	8750	0.131	0.794
6K	5640	4440	140	-	10	-	10,230	0.627	0.980
8F	2230	5240	380	-	110	-	7960	0.342	0.923
4F5	1040	150-	10	-	-	-	1200	0.895	0.988
1F5	3960	980	20	-	-	70	5030	0.835	0.984
2020									
4F	3053	653	387	-	-	-	4093	0.750	0.870
2GM	1013	7547	2307	27	-	-	10,894	0.107	0.721
9F	1440	3853	1373	13	-	-	6679	0.237	0.729
6K	6147	6893	267	-	53	-	13,360	0.537	0.969
6L	5973	2080	480	-	27	200	8773	0.719	0.903
1L	5400	1693	347	-	-	-	7440	0.760	0.934
2L	2173	1720	627	-	347	-	4867	0.481	0.773
17F	1773	240	1280	13	-	-	3306	0.472	0.525
20F	780	-	500	-	-	-	1280	0.524	0.524
FZ19	2947	1307	160	-	-	53	4467	0.711	0.941

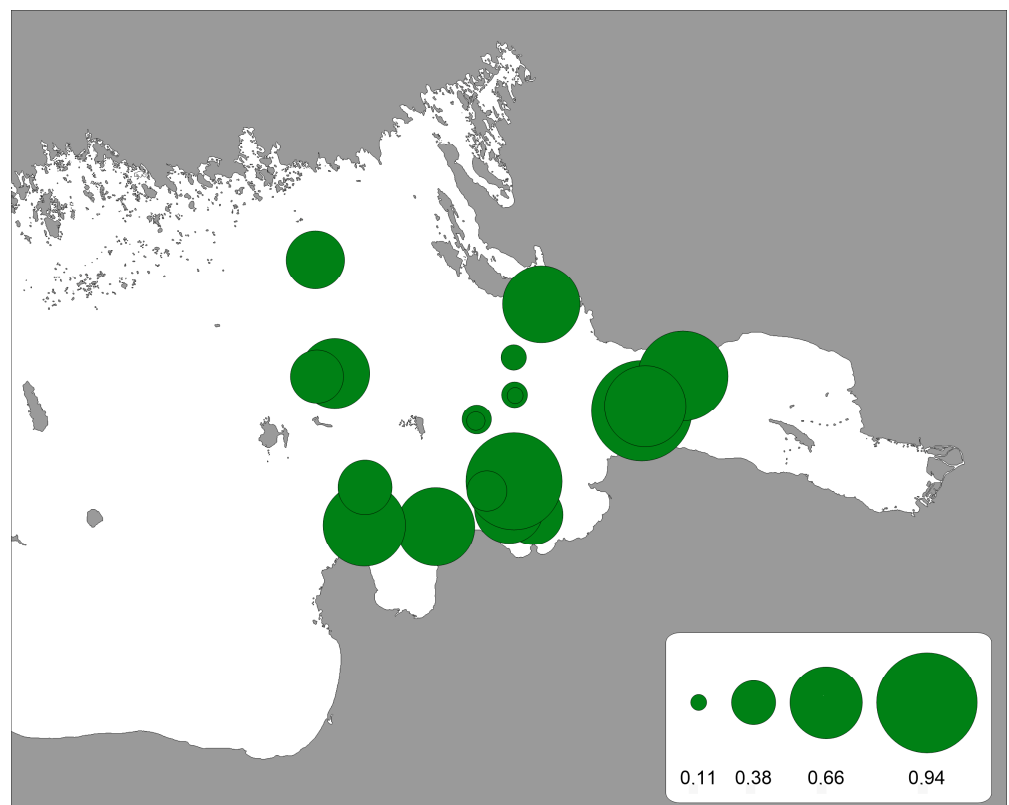


Figure 4. Benthic Opportunistic Polychaetes Amphipods (BOPA) index at sampling sites in the eastern Gulf of Finland.

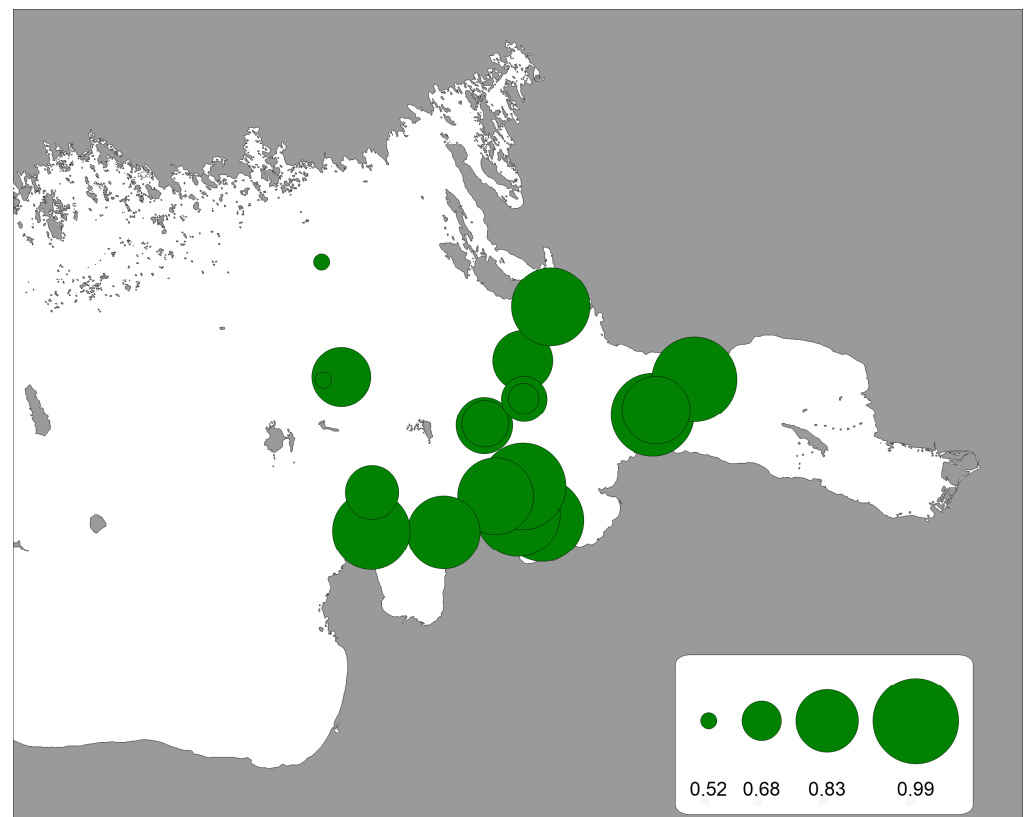


Figure 5. Benthic Opportunistic Annelids Amphipods (BO2A) index at sampling sites in the eastern Gulf of Finland.

3.3. Correlation between Benthic Indices and Environmental Variables

Correlations between the BOPA index and pollution-related variables were low in most cases (Table 2). The significant relationships with cadmium and zinc were only identified. BO2A was correlated with total hydrocarbon, organic carbon, mercury, and cadmium (Table 2).

Spearman's correlation coefficient indicated significant correlations between benthic indices and indices of water and sediment pollution. The correlations between BOPA index and variables of water were positive, resulting in a significant positive relationship with the WP index. The relationship between BO2A and WP was not significant. However, it should be taken into account that oligochaetes were not present at all stations, which led to an underestimation of the index value. If site 20F, where oligochaetes were not found (Table 1), is excluded from the analysis, then the Spearman correlation coefficient between BO2A and WP is +0.67 ($p = 0.003$). Both the benthic indices (BOPA and BO2A) and WP tended to decrease at offshore sites, indicating a better environmental state in the open sea than in coastal areas. In contrast, the correlation between BOPA (BO2A) and SP index was negative. The relationships with the content of contaminants in bottom sediments were also negative, with one exception (BOPA and PAHs).

The abundance of the amphipod *M. affinis* was positively correlated with the concentration of metals: Pb ($p = 0.03$) and Cd ($p = 0.006$). No significant correlations were found for abundances of other species and any environmental variables (Table 2).

Regarding hydrographic variables, Spearman's correlation coefficient showed no significant correlations between BOPA, BO2A and the six analyzed variables (Table 3). Significant correlations were found between values of BOPA, BO2A, abundance, and depth. The abundance of oligochaetes correlated negatively with surface salinity ($p = 0.03$). The BOPA index, WP index, and abundance of *Marenzelleria* correlated negatively with depth, whereas the relationships of the SP index and abundance of *M. affinis* correlated positively

with depth (Table 3). These correlations agree with the mentioned above spatial changes of WP, SP, and BOPA indices in the direction from coastal to offshore sites (Figures 2, 3 and 5).

Table 2. Spearman Rank Order Correlations between BOPA and BO2A indices, benthic species abundance, and pollution-related variables. * $p \leq 0.05$, ** $p \leq 0.01$.

Variable	BOPA	BO2A	<i>Marenzelleria</i>	Oligochaeta	<i>M. affinis</i>
Total HC	0.20	0.58 **	0.34	0.14	−0.44
TP _{wat}	0.29	−0.05	0.14	−0.33	−0.17
Chl-a	0.28	0.11	0.35	−0.12	−0.18
WP	0.47 *	0.42	0.38	−0.28	−0.54 *
PAH	0.18	−0.23	0.19	−0.17	0.13
TOC	−0.20	−0.47 *	−0.18	−0.13	0.27
Hg	−0.10	−0.47 *	−0.23	−0.29	0.25
Cd	−0.51 *	−0.49 *	−0.36	0.29	0.61 **
Cu	−0.26	−0.28	−0.15	0.09	0.34
Pb	−0.43	−0.35	−0.27	0.32	0.49 *
Zn	−0.50 *	−0.33	−0.26	0.37	0.45
TP _{sed}	−0.31	−0.22	−0.01	0.31	0.29
SP	−0.47 *	−0.68 **	−0.30	0.20	0.65 **

Notes. Total HC, TP_{wat} and Chl-a are, respectively, concentrations of total hydrocarbon, total phosphorus, and chlorophyll-a in water; WP is the water pollution index, PAH is concentration of polycyclic aromatic hydrocarbons in sediments, TOC is total organic content, Hg, mg kg^{−1}, Cd, mg kg^{−1}, Cu, mg kg^{−1}, Pb, mg kg^{−1}, Zn, mg kg^{−1} are concentrations of respective metals in sediments, TP_{sed}, mg kg^{−1} is concentration of total phosphorus in sediment, and SP is the sediment pollution index.

Table 3. Spearman Rank Order Correlations, benthic species abundance, BOPA and BO2A index, indices of sediment (SP), and water pollution (WP) indices with environmental variables. * $p \leq 0.05$, *** $p \leq 0.001$.

	Depth, m	T _{surf}	T _{bot}	S _{surf}	S _{bot}	O _{2 surf}	O _{2 bot}
<i>Marenzelleria</i> spp.	−0.767 ***	−0.199	0.069	−0.004	−0.267	−0.106	0.083
Oligochaeta	−0.063	0.397	0.150	−0.497 *	0.004	−0.031	0.129
<i>M. affinis</i>	0.702 ***	0.082	0.252	−0.215	0.005	0.270	0.287
BOPA	−0.569 *	−0.308	−0.122	0.344	−0.116	−0.065	−0.094
BO2A	−0.798 ***	0.105	−0.241	−0.002	0.026	−0.288	−0.261
SP	0.540 *	−0.029	0.265	0.019	−0.070	0.091	0.177
WP	−0.528 *	−0.256	−0.057	0.037	−0.196	0.079	0.047

Notes. T_{surf}, T_{bot}, S_{surf}, S_{bot}, O_{2 surf} and O_{2 bot} are, respectively, surface and near-bottom temperature; salinity and oxygen level.

3.4. Principal Component Analysis

The principal component and classification analysis (PCA) identified close relationships between estimated benthic indices and pollution-related and environmental variables (Table 4). All the extracted components were explained by three main components: Factor 1, Factor 2, and Factor 3, which together explained 63% of the total variance (Table 4, Figure S2).

Factor 1 represented the majority of estimated variables that were closely related to metal concentration in sediments, their pollution index, and pollution index of water. These variables related closely to the abundance of *M. affinis* and values of BOPA index. Values of PAH and hydrological variables (water salinity, temperature, and oxygen) loaded significantly (loadings >0.7) to Factor 2. BO2A index, depth, and abundance of annelids loaded notably to Factor 3 explained around 17% of the total variance.

Table 4. Factor loadings for the Principal components, significant loadings are >0.70, they are marked in bold. The red color shows variables that form clusters determining the oblique factors for hierarchical analysis.

Variable	Factor 1	Factor 2	Factor 3
HC	−0.36	−0.35	−0.43
TP wat	−0.46	0.09	0.67
Chl-a	−0.67	0.28	0.10
WP	−0.80	0.01	0.18
PAH	−0.07	0.88	0.02
TOC	0.02	0.31	0.64
Hg	0.23	−0.01	0.64
Cd	0.86	−0.01	0.10
Cu	0.62	−0.38	0.16
Pb	0.81	−0.35	0.02
Zn	0.75	0.05	0.00
TPsed	0.28	0.21	−0.13
SP	0.83	0.17	0.35
<i>Marenzelleria</i>	−0.34	0.24	−0.63
Annelida	0.32	0.13	−0.74
<i>M. affinis</i>	0.72	−0.01	0.10
Macrozoobenthos	0.48	0.12	−0.63
BOPA	−0.73	−0.05	−0.07
BO2A	−0.46	−0.15	−0.75
Depth	0.40	−0.22	0.79
T _{surf}	0.42	−0.54	−0.34
T _{bot}	0.04	0.98	0.07
S _{surf}	−0.21	−0.49	0.30
S _{bot}	0.11	−0.91	0.01
O _{2 surf}	−0.11	0.62	−0.04
O _{2 bot}	0.02	0.97	−0.15
Eigenvalue	7.07	5.00	4.32
% of the total variance	27.18	19.23	16.62

Notes. See designations in Tables 2 and 3.

4. Discussion

The positive relationship between BOPA and WP indicates that the polychaete/amphipod ratio presumably adequately reflects the environmental state of the water mass in the eastern Gulf of Finland. The abundance of amphipods and polychaetes was highly correlated with depth, resulting in a negative relationship between BOPA and depth. However, the possibility of a direct influence of depth on the benthic indices is doubtful, since for the purposes of this study we used only data from the depth range (27–52 m), which is considered optimal for the Baltic populations of *M. affinis* and species of *Marenzelleria* [15,33,34]. More likely, it is an indirect depth effect due to reduced water pollution in deeper waters. At the same time, BOPA is not correlated with natural factors, such as temperature, salinity, and oxygen. However, the careful unambiguous interpretation of this result is difficult due to the drastic differences in hydrographical parameters between the two years. At present, polychaetes from the genus *Marenzelleria* and the amphipod *M. affinis* dominate in the northern area of the Baltic Sea [35]. Thus, the BOPA index seems to have the potential to be used in the assessment of water quality in the coastal waters of the Baltic Sea.

BO2A is recommended in the low-salinity zones of estuaries, where oligochaetes replace polychaetes as dominant opportunistic species [23]. In the study area, the high abundance of oligochaetes was mainly connected with the incursion of a non-indigenous species *T. pseudogaster* (Figure S1). In contrast to polychaetes, this tubificid oligochaete has no pelagic stage and spreads very slowly (about 1–2 km per year) from one site, where it was first found in the eastern Gulf of Finland in 1995 [15,28]. Therefore, at present, the abundance of oligochaetes strongly depends on the presence or absence of *T. pseudogaster* at the site and the stage of its invasion process. Based on the Spearman rank correlation and

PCA analysis, the BO2A index was not related to water pollution. PCA demonstrated that BO2A, annelids, and depth were related to PC 3 and with each other (Table 4). Nevertheless, BO2A was positively correlated with WP at sites where oligochaetes were present. Most likely, BO2A can be successfully used after the completion of the invasion when the population of *T. pseudogaster* will stabilize. In addition, BO2A potentially can be used in almost fresh shallow waters of the Gulf of Finland, where polychaetes are absent and Oligochaeta are the main opportunistic taxon. This is especially promising, since in recent years there has been an increase in the number and abundance of amphipods in the littoral zone of the eastern Gulf of Finland due to the invasion of new species [36].

The BOPA, BO2A as well as amphipod abundance were positively related to the SP index and with some trace metals (Cd, Zn, Hg, and Pb). This contrasts with the common view of *M. affinis* as a sensitive species and an indicator of good environmental conditions in the Baltic Sea (e.g., [3]). However, it would be an oversimplification to consider the benthic community solely as just an indicator of environmental changes. Many macroinvertebrates are well-known habitat modifiers that function as ecosystem engineers by modifying their environment, including chemical characteristics of bottom sediments [37,38].

The amphipod *M. affinis* is a deposit-feeder, which ingests particles almost exclusively on the bottom surface from 0 to 0.5 cm [39], reworking the top layer of the sediment intensively. In the Northern Baltic Sea, the soft-bottom surface sediment frequently consists mainly of fecal pellets of amphipods [40]. The laboratory experiments indicated an increased accumulation of cadmium in the sediment when *M. affinis* is present [41,42]. Authors suggest that bioturbation caused by *M. affinis* leads to a greater adsorption of Cd²⁺ to particles, perhaps by stimulating microbial activity. The production of fecal pellets from *M. affinis* could also contribute to higher concentrations of cadmium, since it is possible that there is some excretion of cadmium through the gut [41].

Other members of macrobenthic communities were not significantly correlated with any contaminants (Table 2). The difference may be attributed to the different feeding and burrowing behavior of dominant benthic species.

Tubificid oligochaetes are conveyor-belt feeders. They feed by ingesting at 2–5 cm depth in sediments and deposit feces on the surface of the sediments [43,44]. The permanent deposition of fecal pellets to the surface of the bottom because of tubificid feeding causes the downward migration of the sediment–water interface (e.g., [45]). Thus, the organic-rich and more polluted top layer becomes buried as a result of tubificid activity. On the other hand, bioturbation by oligochaetes increases Cd flux into the sediments [46].

Marenzelleria spp. are bioirrigators, whose activity increases the depth of oxygen penetration into sediments, resulting in the formation of a thicker (up to 10 cm for *Marenzelleria arctica*) oxidized layer [47]. However, the burial and subsequent oxidation of organic matter can counteract the irrigation effects of *Marenzelleria* spp. [48]. The chemical evolution of metals in sediments is regulated by an interrelated suite of redox reactions that mineralize the labile organic matter. Metals are either directly involved in these biogeochemical reactions (e.g., Fe and Mn) or interact with the reaction products (e.g., iron and manganese oxides/hydroxides, and sulfide) via adsorption and/or co-precipitation [49,50]. Bioirrigation of anoxic sediment by polychaete modifies the redox conditions and, consequently, the repartition of many metals due to their redox sensitivity. In the eastern Gulf of Finland, the higher polychaetes abundance coincides with lower content of metals Fe and Mn in the solid phase, apparently due to the more intensive use of oxides/hydroxides of these metals in the organic matter oxidation [51]. Similarly, in the experiments, Fe and Mn leakage strongly increased in the bioturbated sediments with *Marenzelleria arctica* [52]. However, while many studies have focused on the diverse biogeochemical consequences of the *Marenzelleria* spp. invasion to the Baltic Sea [53–56], the response of trace metals to the polychaetes activity is still not understood. Our data indicate that *Marenzelleria* activity tends basically to decrease the content of all metals in the solid phase of sediments. However, these effects were weak and not statistically significant (Table 2).

Although we were the first to use BOPA and BO2A indices in the Baltic Sea, the Polychaetes/Amphipods ratio has proved successful in many geographic locations for detecting the effect of different anthropogenic pressures [23]. Nevertheless, some authors, such as Equbal et al. [57], have stressed that the sensitivity of BOPA is low and fails to detect any changes in benthic composition under the influence of disturbances (anthropogenic or natural). Similarly, in the Ebro estuary (Iberian Peninsula), the Spearman correlation analysis revealed that BOPA was negatively correlated with Pollution Pressure [58]. The authors suggest that the probable explanation is that BOPA was essentially developed to assess hydrocarbon spill impact over benthic invertebrate communities; in the way that amphipods, the main component of BOPA, are recognized to be sensitive to hydrocarbons [58]. We, however, did not find any relationship between amphipod abundance and concentration of hydrocarbons in both water and sediments. Equbal et al. [59] concluded that the detection of stress gradients by BOPA can only occur when there is high numerical abundance of opportunistic and sensitive species. In addition, the sensitivity of the BOPA index seems to depend on the geographic location, community structure, and the species-specific responses to different sources of contaminants and the nature of disturbances [23,57].

5. Conclusions

The results of this study demonstrate that the BOPA and possibly BO2A ratios are generally positively correlated with the water quality in the Gulf of Finland, and consequently have the potential to be used in the assessment of the coastal waters in the Baltic Sea and in low salinity waters more generally. However, the concentration of contaminants in sediments seems to be strongly influenced by the activity of the benthic organisms themselves, specifically the amphipod *M. affinis*.

This is important for the correct interpretation of data from monitoring programs. Firstly, amphipods and annelids are frequently classified as respectively sensitive and opportunistic species and are used for the calculation of other benthic indices, such as AZTI, BQI [3,11]. Our results indicate that BOPA and BO2A ratios, as well as, presumably, other benthic indices, should be applied with caution in assessments of sediment quality.

Secondly, when monitoring harmful substances, it is necessary to take into account the possible effect of benthic macroinvertebrates on the environment. For example, a significant decrease in the concentration of cadmium in the bottom sediments of the Gulf of Finland during the few last decades [28,60] in the context of our results can be explained not only by the trend towards a decrease in the supply of metals from the watershed, but at least partly by a change in bottom communities, namely a sharp decrease in the abundance of *M. affinis* in the Gulf of Finland (as a result of the hypoxic events of 1996 and 2003) and subsequent *Marenzelleria* spp. invasion [15,28,61]. Further field and laboratory studies are needed to assess the real interrelationships between benthic invertebrates and concentration of trace metals and other hazardous substances in the sediments.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jmse11010190/s1>, Table S1: Hydrographic conditions at study sites; Table S2: Standardized values of pollution related variables; Figure S1: Percentage of different oligochaete species in the total abundance of oligochaetes; Figure S2: Factor loadings for the first three principal components.

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