



# Improve EIA methodology in aquaculture applying biotic indices

Bætt vöktun fiskeldis með notkun líffræðistuðla

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#### ABSTRACT

## English

Biotic indices were tested in Icelandic conditions by applying four different biotic indices on several benthic samples collected between the years 2016-2017 in the Westfjords. Index applicability was investigated and index performance was tested by looking at the relationship of the index's values with redox potentials measured in the sediment sample. Results did show positive correlation for some indices. M- AMBI showed best performances but other biotic indices did not exceed the performance of the diversity index (Shannon-Wiener), supporting the assumption that redox potential is un-reliable on its own to conclude which index perform better.

## Íslenska

Líffræðistuðlar voru prófaðir við íslenskar aðstæður. Fjórum ólíkum stuðlum var beitt á niðurstöður greininga sýna sem tekin voru af botni fiskeldissvæða á Vestfjörðum á árunum 2016 og 2017. Skoðað var hvort vandkvæði væru við að beita líffræðistuðlunum á íslensk gögn. Þá var frammistaða stuðlanna skoðuð með því að bera niðurstöður þeirra saman við niðustöður redox efnamælinga sömu sýna.

Niðurstöður sýndu jákvæða fylgni fyrir suma stuðlanna en einungis M-AMBI stuðullinn hafði meiri fylgni við niðurstöður redox mælinga en fjölbreytileikastuðullinn Shannon- Wiener. Þessar niðurstöður benda til þess að hugsanlega sé ekki nægilegt að nota fylgni við redox mælingar til að segja til um hvaða líffræðistuðull henti best við íslenskar aðstæður.

#### INTRODUCTION

Aquaculture is a growing sector in Iceland. Accumulation of organic matter and chemical dispersion in the water column are some of the impacts this activity has on the surrounding environment. Organic enrichment of bottom sediments leads to a surplus demand for oxygen by bacterial communities, in order to degrade organic matter, which eventually leads to anoxia and sulphide production and therefore changes in animal communities. Environmental Impact Assessment (EIA) done to assess organic enrichment is regulated in Iceland by ISO 12878 standard. Minimum required parameters are redox potential at the bottom and animal community analyses on predetermined sites based on bathymetry and sea current.

Benthic communities (species diversity and abundances) found in sediments are used in the calculation of indices. Indices are used to express the quality status of sediments summarized in a numerical value. For the past years, the Shannon-Wiener diversity index applied on the benthic community has been widely used in order to qualify the sediment status and therefore the degree of pollution. This index, which was not developed specifically to assess pollution, does not differentiate between species and their specific resistance to pollution and could eventually give misleading results. Different species react differently to physico-chemical changes at the sea bottom and decreased oxygen availability. In this regard more appropriate indices have been developed recently. Because they take into consideration the specific resistance of different species to a pollution gradient in their calculation, they are called biotic indices. Their use is becoming more and more common and has been implemented by FAO, EU (Water Framework Directive 2000/60/EC), Aquaculture Stewardship Council (ASC) standard and the above-mentioned ISO 12878:2012. Though to date there is not consensus regarding which of these indices is the best.

In Iceland one of those indices has been used experimentally on sewage discharged on a masters project (Gharibi Arastou, 2011). Indices have never been applied on aquaculture monitoring until recently (after this grant was granted) in Laugardalur (Tálknafjörður) in an EIA conducted in 2017 (Velvin and Gunnarson, 2017).

The goal of this project was to investigate the applicability (qualities, faults and open issues) and eventually establish performances by applying four different indices on benthic samples obtained from several mariculture sites in the Westfjords part of Iceland (Gallo Cristian, 2017 and 2018, Gallo Cristian and Margrét Thorsteinsson, 2017 a,b,c). Applied indices are all intended for soft-bottom conditions, they were selected after literature review and considerations of this author. The applied

biotic indices were: M-AMBI Marine Biotic Index described in Muxika et al. (2007) based on AMBI index (Borja et al., 2000), Invertebrate Species Index (ISI) described in Rygg (2002), Benthic quality Index family (BQIf) described in Dimitriou et al. (2007) based on BQI index (Rosenberg et al., 2004) and Norwegian Sensitivity Index (NSI) described in Rygg and Norling (2013). Infaunal Trophic Index (ITI) after Word (1979) present in the grant application was abandoned for a degree of impracticability found by other authors (Maurer et al. 1999, Diaz et al. 2004).

The redox potential measured on top 2 cm on the same samples was used as a reference parameter for correlation tests, which were intended to point out which of the four indices performs best. Redox potential was chosen, as it is the only chemical parameter viable at this stage as demanded by the ISO 12878 standard.

Results of the study could strengthen the current methodology used to evaluate environmental effects of aquaculture by making evaluation more accurate. If these indices will give more accurate *"vision"* on the situation of the sediment on sea bottom, the management of aquaculture with the purpose of minimising environmental effects should become more effective.

#### METHODOLOGY

Changes in benthic community due to organic enrichment cause by aquaculture are based on the benthic community succession paradigm described first in Pearson and Rosenberg (1978). Pearson and Rosenberg argued that unidirectional stress caused by an environmental disturbance will affect individuals, populations, and communities according to the intensity of the stress. The first response to environmental stress is adaptation by an individual within its abilities to respond. At some point, the organism is no longer able to respond to the stress, and it will then be replaced by another better adapted individual. Beyond this level the species will be replaced by a group of species better adapted to the new conditions. Based on this pattern, it is important that tolerance should be analysed at the species level or lowest possible taxonomic level, as species within the same genus may show great discrepancies in tolerance (Pearson and Rosenberg, 1978).

All indices applied here are based on the concept of "sensitivity": species more sensitive to the organic accumulation will eventually disappear from the impacted area, and the more sensitive the species is, the shorter it will take for that species to disappear. According to this, species that occur in

high-diversity samples are classified as sensitive species. Species found in low-diversity samples are instead usually classified as tolerant or resistant. Presence of many sensitive species in a community indicates a healthy environment.

#### M-AMBI

AMBI is a marine biotic index developed for soft-bottom benthos of European estuarine and coastal environments (Borja et al. 2000). It has been tested first in the Bay of Biscay but was further adjusted with datasets from other EU countries, Intercalibration Working Groups (Borja et al., 2006). It ascribes each species to an ecological group according to its sensitivity to an increasing stress gradient providing a continuous range between 0-6 (semiquantitative scale). Assignments are partly based on expert judgment (subjective evaluation). The index is based upon the percentages of abundance of each ecological group according to the formula:

AMBI index= {(0 x % GI) + (1,5 x % GII) + (3 x % GIII) + (4,5 x GIV) + (6 x GV)} / 100

Species not assigned to a group were not taken into account. Values obtained represent quality of bottom conditions in a discrete range from 0 (unpolluted) to 7 (extremely polluted).

The M-AMBI rapresents a further development of the AMBI index. By combining the AMBI with Shannon Wiener index, species density, biomass and richness, the index is intended as an objective tool in assessing ecological quality status (Muxika et al., 2007). Both AMBI and M-AMBI are widely used in EIA in aquaculture, expecially in the EU according to WFD. Application of the index can be done directly by using a specific software available at www.azti.es.

#### ISI

Development of the Indicator Species Index (ISI) was based on datasets from 1080 Norwegian softbottom fauna samples (Rygg, 2002). The species list was updated by the same author in 2012 (3200 samples involving 1153 stations). It found 1882 taxa but only 591 of them (87% of individuals) were found in more than 20 samples, which was required for sensitivity value assignments in ISI 2012. The methodology is described in Rygg (2002) and Rygg and Norling (2013).

Among the samples in which a taxon occurred, the five samples having the lowest ES100 values were selected and their average ES100 calculated. The diversity index ES100 (Hurlbert 1971) of the sample was selected as an indicator of the stress level endured by the species in the sample. The average of the five lowest ES100 was defined as the sensitivity value of that taxon. Taxa in some cases were aggregated into one wider unit (taxon) and the sensitivity of the taxon as a whole was established.

Sensitivity values were so forth calculated for 200 taxa. The ISI index of a sample was defined as the average of the sensitivity values (ES100min5) of the taxa occurring in the sample and calculated by using an appropriate formula. Species that occur in the sample, but have no sensitivity values assigned to them, are ignored in the calculation of ISI. Only presence/abscence of the species, not their abundance, is considered. The index is calculated according to the formula:

$$ISI = \sum_{i}^{S} \left[ \frac{ISI_{i}}{S_{ISI}} \right]$$

ISIi is the sensitivity value of species i,  $S_{ISI}$  the number of species with assigned values.

## BQIf

Benthic quality index (BQI) is based on soft bottom Swedish dataset collection (4676 samples from 257 stations collected between 1969-2002) (Rosenberg et al., 2004). Calculations for species specific sensitivity were based on Hulbert's 1971 formula as for ISI index. The site BQI combines the Hulbert index values, made among 50 individuals (ES50), with the species abundance distribution along a gradient of disturbance, and the total number of species at that site. Using ES50 instead of ES100 allows inclusion of samples with abundances between 50 and 100 in the analysis, which could be useful in disturbed areas. The most tolerant individuals of the species are likely associated with the lowest ES50 value. The developers selected 5% of the population as the species tolerance value (ES50<sub>0,05</sub>). The index is then calculated according to the formula:

$$\mathbf{BQI} = \left(\sum_{i=1}^{n} \left(\frac{A_i}{\operatorname{tot} A} \times \operatorname{ES50}_{0.05i}\right)\right) \times {}^{10} \log(S+1)$$

Using the mean relative abundance (A) of *i* species puts weight on common species in relation to rare species. Using the mean number of species (S) at the station gives more weight to diversity, as high species diversity is related to high environmental quality.

This index was upgrade in Leonardsson et al. (2009). The sampling used for the development of the index extends until the year 2005. Upgrades include a multiplied abundance factor N/(N+5) in order

to reduce the influence of few individuals on stations with poor environmental conditions. The upgraded index is calculated by the formula:

$$BQI = \left[\sum_{i=1}^{S_{classified}} \left(\frac{N_i}{N_{classified}} * Sensitivity \ value_i\right)\right] \\ * \log_{10}(S+1) * \left(\frac{N_{total}}{N_{total}+5}\right)$$

where *S* classified is the number of taxa having a sensitivity value, *Ni* is the number of individuals of taxon *i*, *N* classified is the total number of individuals of taxa having a sensitivity value, the sensitivity value *i* is the sensitivity value for taxon *i*, *S* is the total number of taxa, and *N* total is the total number of individuals in the sample ( $0.1 \text{ m}^2$ ). Taxa not given a sensitivity value are excluded from the sensitivity factor but included in the total number of species and abundance factors when calculating BQI.

In the calculation of BQIf, family (f) is based on the BQI index and calibrated with Shannon diversity index (H'), AMBI and BENTIX to maximize the consensus on benthos sensitivity (Dimitriou et al., 2012). This index calculated the sensitivity values at a higher taxonomic level, family, instead of species (lowest taxonomical level). The concept of "taxonomic sufficiency" was first developed by Warwick (1986), it was then investigated by several reserchers (Dimitriou et al., 2012), and it was finaly confirmed as best cost-benefit balance between the time and effort required for the analysis and the accuracy obtained (Karakassis and Hatziyanni, 2000). Using family brings convenience, due to the lack of need for a skilled benthos analyser, decresed time for the analysis, and lack of possible misidentifications of species. The sensitivity values (ES50<sub>0,05</sub>) were calculated for 260 benthos families from a dataset of 1010 samples and ecological status threshold limits for BQIf in Dimitriou et al. (2012). The values calculated for the BQIf indicator are significantly and highly correlated (p < 0.0001) to those calculated for all the above-mentioned indicators and it provides judgment on ecological status close to their average (Dimitriou et al., 2012).

#### NSI

Norwegian species-sensitivity based index (NSI) was developed in Norway in 2013 (Rygg & Norling, 2013). The datasets used are from samples collected in Norwegian fjords and coastal waters between 1980 and 2011. A total of 3200 samples from 1153 stations were used in the calculations. There were 1882 taxa analysed, but 591 of them (87% of total individuals) were found in more than 20 samples, which was required for sensitivity value assignments in NSI, as in ISI2012. NSI is a quantitative index, using the species abundances to weight different species sensitivities (ES100) in the calculations. Each

individual of each species was assigned the ES100 (Hurlbert 1971) value of the samples in which it occurred. The sum of all ES100 values for all individuals of each species was then divided by the total number of individuals of each species to obtain the ES100 average value, defining the sensitivity value (ES100avg) of the species. The NSI sensitivity index value of a sample is obtained by dividing the sum of ES100avg values of all individuals in the sample, by the total number of individuals in the sample, giving the average species sensitivity value of all individuals in the sample. Only the species with an ES100 value assigned to them are to be included in the calculation. The NSI index is calculated with equal weight given to each individual according to the formula:

$$\text{NSI} = \sum_{i}^{S} \left[ \frac{N_i * NSI_i}{N_{NSI}} \right]$$

N*i* i individuals of species *i*, NSI*i* is the sensitivity value of species *i*, N<sub>NSI</sub> the number of individuals with assigned values.

#### **Correlation test**

A Spearman and Pearson correlation test were applied to the values of the four indices and the chemical redox potential value acquired on sediment during sampling on the same sample. R studio 3.4.0 (R core team, 2017) was used for the test. The test was applied first to all stations and later to only stations with a species/taxa abundance between 6-14, as that is considered the critical range of values that eventually set the line for management measures and restoration.

## RESULTS

Benthic communities and abundances are given in Appendix 1. Results of index calculations are given in Appendix 2. Quality classes assigned to sediment condition based on calculated indices (High-Good- Fair- Poor and Bad) for each station, according to different index categorization limits based on increasing redox values are given in table 1.

Table 1. Quality status of sediment found in different stations according to Eco-status limits, developed independently for each index. Arrangement of stations is done according to increase Redox (Eh) value measured in the sediment, which is used as reference value for the correlation test.

Stations	Redox Eh (mV)	M-AMBI	ISI	NSI	BQIf
LauD	-142	Bad	Poor	Bad	Poor
HauA	3	Bad	Bad	Bad	Fair
HauF	8	Poor	Poor	Bad	Poor
HlaH	22	Poor	Poor	Poor	Poor
HauB	27	Fair	Fair	Poor	Fair
HlaG	39	Bad	Bad	Bad	Bad
MosD	55	Fair	Poor	Poor	Poor
HlaF	65	Bad	Bad	Bad	Bad
MosB	67	High	Good	Fair	Good
HlaD	74	Fair	Fair	Poor	Fair
MosA	84	Good	Good	Fair	Good
HlaE	93	Fair	Fair	Poor	Good
HauH	112	High	Good	Fair	Good
LauC	113	Bad	Poor	Bad	Poor
HauE	129	High	Good	Fair	Good
HlaA	144	Bad	Poor	Bad	Poor
HlaB	154	Poor	Fair	Bad	Poor
HauC	163	High	Good	Fair	Good
MosK	166	Good	Good	Fair	Good
Hlal	170	Good	Good	Good	Fair
Haul	171	High	Good	Fair	Good
HauG	173	High	Good	Fair	Good
HauD	179	High	Good	Fair	Good
TjaD	183	Poor	Fair	Poor	Bad
LauB	186	Good	Fair	Fair	Good
HlaC	208	Fair	Fair	Poor	Fair
MosC	317	Good	Fair	Fair	Good

Evident anomalies between redox potential and sediment quality classes were present at MosB, LauC, HlaA, HlaB, and TjaD.

Spearman correlation values between each index and redox measurements are in Table 2. All indices were significantly correlated to the redox measurements except for NSI. M-AMBI had the strongest correlation with a correlation coefficient of  $r_s$ = 0,607 and *p*-value= 0,0005. Shannon-Wiener index had the second-strongest correlation, and ISI had the lowest correlation.

Table 2. Spearman correlations values ( $r_s$ ) and *p*-values between indexes and redox mesurments. Italics indicates statistical significance (p < 0.05).

Index	r <sub>s</sub>	p -value
M- AMBI	0,607	0,0005
Shannon - Wiener	0,541	0,0024
BQI f	0,500	0,0058
ISI	0,460	0,0119
NSI	0,052	0,3643

Correlation test was applied to benthic samples with number of taxa ranging between 6 and 14. Pearson's test gave all p-values bigger than 0,05 pointing out no correlation between redox potential and any of the indices (table 3).

Table 3. Pearson correlations values (r) and p-values between indexes and redox mesurme	nts.
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Index	r	p -value
Shannon - Wiener	-0,005	0,991
BQI f	0,009	0,982
M- AMBI	0,029	0,947
NSI	0,220	0,600
ISI	0,582	0,130

#### DISCUSSION

Results of the correlation test based on redox measurements show that Shannon-Wiener diversity index performs better than most of the new tested biotic indices and therefore seems to not support our hypothesis regarding a need for a benthos sensitivity-based index in order to improve the EIA. M-AMBI shows promising correlation results. This index is based on a subjective index (AMBI) but itself includes the Shannon-Wiener index as a means of objectivity. Other applied indices which are fully objective, chosen because they were developed in Norway and therefore reasonably more like to Icelandic conditions, did show less or no correlation, as in the case of NSI index.

NSI showed correlation with AMBI according with the developers, and NSI seems to perform better than AMBI in Norwegian fjords, showing a better correlation with different pressures compared to AMBI (Rygg & Norling, 2013). High correlation has been found elsewhere between BQIf (BQI) and AMBI (Dimitriou et al., 2012).

Biotic indices developed in accordance with pressure gradients should, in theory, perform better than a diversity index which does not take specific tolerance into consideration.

Reasons for these ambiguous results could be found in the resolution of the redox measuring device (60mV) or size of the sampling area. Samples, taken during the EIA, considered in this research were collected with a 250 cm<sup>2</sup> grab size rather than 0,1 m<sup>2</sup>, which was used for the development of the biotic indices applied. Other explanations could be found in the species/taxa that were excluded or moved to a higher taxonomical level because sensitivity values were not given by the index's developers. Species/taxa found in the samples that were excluded or re-assigned, due to no assigned sensitivity values, could eventually affect the values of calculated indices and therefore their reliability. Taxa with missing sensitivity values rapresent limitation for the application of benthic indeces and was one of the reasons why the BQI original index was not applied.

Investigation into this possibility showed that for AMBI (version June2017), the taxa without assigned values were Amphipoda, *Eubranchus sp., Eudorella sp., Mammiphitime cosmetandra*, Oedicerotidae and *Pleurogonium sp.*. These taxa corresponded to 5,3 % of individauls for the station named MosK, 4 % for HauD, 2,4 % for LauB, 2,1 % for HauG, 1,4% for HauH and HlaC, 0,9% for LauA, 0,8% for HauE and HauI,0,7% for HlaD, 0,4% for HlaB and 0,2% for HauD and HauC. The rest of stations had 100% of assigned values.

Instead of excluding the species, the ISI and NSI indices (which are based on the same specie sensitivity list, but not same value) offer the possibility to move them to a higher taxonomical level. The percentage of individuals that needed to be re-assigned were: 21% for station MosA, 20% for MosD, 17% for HauD, 14% for HauA and HauF, 13% for HauI, HIaB and LauD. All other stations had less than 10% re-assignment. The taxa *Sternapsis scutata/islandica* and *Parougia nigridentata* were re-assigned as Polychaeta, *Microphthalmus aberrans* was re-assigned as Hesionidae. Re-assigned taxa were relatively common in the samples and their re-assignment represents an important issue for the application of this index.

In the calculation of the BQIf index, taxa such as Amphipoda, *Eubranchus sp., Lepeta caeca*, Nemertea, Oligochaeta, Oedicerotidae, *Pleurogonium sp., Stenosemus albus* and *Yoldia hyperborea* had un-assigned sensitivity values and therefore had to be included in the calculations as mentioned in the formula. The percentage of individuals included in those taxa were 3% in station HauD and MosC, 2% in HlaC, LauA, LauB, 1% in HlaD, HlaI and MosK.

According to these percentages, the number of species/taxa without assigned sensitivity values does not have a relevant impact on the index values. Considering that for ISI and NSI the taxa were reassigned rather than excluded seems to exclude strong implication on the results for this reason.

Correlation values calculated for the middle-class samples (with number of taxa between 6-14) did not show more promising results. Number of samples were few and statistical test was affected by this matter.

According to these findings, I should conclude that M-AMBI performs best in Icelandic conditions. Based on the discussion above, this author cannot confidently state that these findings are conclusive enough to exclude the possibility that other benthic indices could perform better. Norwegian monitoring guidelines suggest considering more than one index, with increases costs and allows for uncertainty in decision making processes. We must conclude that the redox parameter, on which we based our correlation tests, is probably not reliable, or at least not on its own, to draw satisfactory conclusions.

More parameters will therefore need to be considered in order to assess which biotic index perform better in Icelandic conditions. Total organic carbon (TOC), free sulphide (H<sub>2</sub>S), dissolved oxygen (DO), total phosphorus (TP) and total nitrogen (TN) might be options. Those parameters are, however, not

currently compulsory in EIA in Iceland, and therefore extra effort will need to be put into testing their viability in the pursuit for the most reliable index in Icelandic conditions.

APPENDIX 1. Benthic communities and abundances for five mariculture sites in Westfjords (Iceland).

Tritia incrossata Yoldia hyperborea	Thyasira flexuosa	Tanaidacea Terebellides stroemii	Stemaspis scutata Syllis sp.	Spionidae	Spio goniocephala	Scolopios armiger Sinunculidae	Scalibregma inflatum	Prionospio steenstrupi Sabellides borealis	Praxillella spp.	Praxillella praetermissa	Palvdora sab.	Platyhelminthes	Phyllodoce maculata	Photis spp.	Pholoe minuta	Pectinaria spp. Pherusa falanta	Pectinaria koreni	Parougia nigridentata	Owenia fusiformis Paraediceras lunceus	Ophryotrocha labifera	Ophryotracha spp.	Ophiura spp.	Olgochaeta	Oediceropsis brevicornis	Nuculana spp.	Nuculana minuta	Nereis spp.	Nereimyra punctata	Nephtys spp.	Nephtys caeca	Nematoda	Microphthalmus aberrans	Melinna cristata	Mammiphitime cosmetandra Mediomastus fragilis	Maldane sarsi	Malacoceros fuliginosus	Lysianassidae	Lunatia pallida	Levinsenia gracilis	Leucon nasicoides	Lepeta caeca	Ischnochiton albus	Harmothoe spp.	Goniada maculata	Galathowenia oculata	Exogone sp.	Eudorella emarginata Fudorella sn	Euchone sp.	Euchone analis	Eteone longa	Ennucula tenuis	Dorvilleidae	Cossura longocirrata	Cirratulus cirratus	Chaetozone setosa	Capitella capitata	Brachydiastylis resima	Astarte borealis Asteroidea	Aricidea suecica	Amphipoda	Ampharetidae Amphineis aunneri	Ampeliscidae	Abra nitida
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Station	Index					Redox (r	nV)
	Shan-Wie loge	M-AMBI	ISI	NSI	BQIf	Redox	Redox Eh
HauA	0,48	0,15	4,26	9,39	7,79	-213	3
HauB	1,44	0,43	7,21	12,25	7,59	-189	27
HauC	2,44	0,83	7 <i>,</i> 85	21,14	13,16	-53	163
HauD	2,86	0,86	7,96	21,63	15,32	-37	179
HauE	2,34	0,82	7,75	22,84	14,03	-87	129
HauF	0,53	0,23	5,15	9,46	3 <i>,</i> 96	-208	8
HauG	2,96	0,91	7,81	21,28	14,79	-43	173
HauH	2,95	0,93	7,95	22,70	15,62	-104	112
Haul	2,68	0,89	8,62	22,84	15,11	-45	171
HlaA	0,20	0,18	6,10	7,50	3,46	-74	144
HlaB	0,50	0,25	6,76	9,48	4,22	-64	154
HlaC	1,40	0,48	6,88	11,15	6,47	-10	208
HlaD	1,40	0,45	7,33	13,95	6,45	-144	74
HlaE	1,60	0,51	6,77	17,40	10,73	-125	93
HlaF	0,10	0,16	4,03	7,19	1,56	-153	65
HlaG	*	0,01	1,58	6,98	0,68	-179	39
HlaH	0,90	0,23	5,35	13,36	2,25	-196	22
Hlal	1,40	0,57	7,85	23,59	10,09	-48	170
LauB	2,20	0,69	6,89	21,88	13,18	-32	186
LauC	0,69	0,18	4,60	10,18	2,94	-105	113
LauD	0,58	0,15	5,35	10,03	2,49	-360	-142
TjaA	*	0,13	7,50	22,16	0,96	-340	-122
ТјаВ	1,57	0,45	6,20	20,08	5,71	-360	-142
TjaD	0,95	0,29	6,40	11,04	1,88	-35	183
MosA	1,82	0,59	7,53	14,17	14,59	-134	84
MosB	2,62	0,79	7,97	21,09	13,91	-151	67
MosC	2,70	0,76	6,81	19,97	14,10	99	317
MosD	0,92	0,40	5,63	11,12	3,81	-163	55
MosK	2,79	0,69	7,52	22,07	13,34	-52	166

**APPENDIX 2**. Results of indices calculations (four biotic index) and Shannon- Wiener, and redox potential values measured on bottom sediment during sampling.

\*Value could not be calculated because of only one specie/taxa present.

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