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Assessment of the ecological impact of an oil spill on shallow brackish-water benthic communities: a case study in the northeastern Baltic Sea

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Abstract. The relationship between the abiotic environment and the structure of macrozoobenthos was studied in shallow bays of the southwestern Gulf of Finland, the Baltic Sea, that were impacted by a moderate oil spill in 2006. Seabed sediment characteristics, depth, and wave exposure best explained the structure of benthos. Statistical analyses did not show explicit effects of oil on benthos. The decrease in the abundance of *Bathyporeia pilosa* and *Macoma balthica* may be related to the oil, but it would be speculative to attribute this pattern solely to the spill without a proper before-after-control-impact (BACI) design with several control locations. Our study clearly showed that in the case of accidental environmental impacts like oil pollution it is impossible to apply a proper setup of the BACI design. This leads to difficulties in distinguishing between the effects of natural environmental factors and oil on the biota. The study also advocates for needs of alternative methodologies in order to effectively assess the impacts of accidental anthropogenic disturbances on benthic communities.

Key words: Baltic Sea, benthic invertebrates, natural disturbance, oil spill.

INTRODUCTION

The spatial and temporal distribution of species and communities is largely driven by both natural and anthropogenic forcing. Benthic invertebrates and macrophytes are known to be indicative of various disturbances as they are spatially and temporally stable and different species exhibit varying tolerance to environmental factors (Gray et al., 1990; Kotta et al., 2004; Torn et al., 2006; Lauringson et al., 2012). The use of benthic macroinvertebrates and macrophytes in the assessment of the ecological status of coastal waters is legislated and mandatory in the European Union by the requirements of the Water Framework Directive (Borja et al., 2009).

One of the key problems is how to distinguish between natural and anthropogenic disturbances and quantify their relative importance. A before-after-control-impact (BACI) design is a common approach to distinguish the effects of discrete anthropogenic disturbance events. Underwood (1992) criticized the usual BACI design because it has only a single control site and proposed a ‘beyond BACI’ design, which has several control locations. However, BACI and beyond BACI designs can be properly applied only if the timing and location of disturbance are known prior to the disturbance event. In the cases of unintended accidental disturbances such as oil spills, a proper BACI design cannot be applied and subsequent distinguishing between natural and oil-induced changes is complicated (Queiroz et al., 2006). Moreover, economic and logistical constraints pose limitations on the sampling extent and frequency. Thus, provided a high natural variability of communities, most environmental impact assessments can reveal only very acute effects (Kraufvelin, 2000).

Oil spills potentially affect benthic communities in many ways, for example through modification of habitat characteristics, suffocation and/or poisoning of flora and fauna, and removal of the key habitat forming species that may indirectly affect other components of benthic life (Baker, 2001). Oil transportation is significantly growing in the Baltic Sea and so is the number of shipping accidents (HELCOM, 2010), posing an increasing threat to the natural communities.

An oil spill was detected in the southwestern Gulf of Finland in January 2006. Severe storms hindered the removal of the oil from the sea surface. It was estimated that approximately 10 tonnes of heavy oil stranded to the shores of the Keibu Bay area, northwestern Estonia. Samples for macrobenthos were collected a few months after the pollution and in 2007 and 2009. Data collected in the area in 1997 were available for reference. It is plausible, though, that the nine years gap between the reference and impact data is too long for adequate comparison due to natural changes in benthic communities. In addition, as the timing and location of oil pollution were not known in advance, a proper setup of sampling stations and reference areas was not possible. Considering these complications we aimed (1) to assess the interannual differences in the benthic community to reveal the possible effect of oil and (2) to evaluate the contribution of environmental factors including oil to the variability of benthic communities. We expected that if oil is an important driver of change in benthic invertebrate assemblages, the signal of an oil spill event has to be present in the spatial pattern of biological data in the year of the oil spill. We also expected that other environmental variables modulate the strength of the impact of the oil spill on the biota.

MATERIAL AND METHODS

Keibu Bay (59.25° N, 23.67° E) is situated in the southwestern Gulf of Finland (Fig. 1). The area is highly exposed to the sea. The prevailing depths remain between 5 and 20 m, salinity is between 6 and 8, and bottom deposits consist mainly of fine to medium sands. Hard bottoms, consisting of pebbles and boulders, are located in the vicinity of peninsulas and cover a small area. Benthic vegetation is scarce in the area and is mainly found on hard substrate.

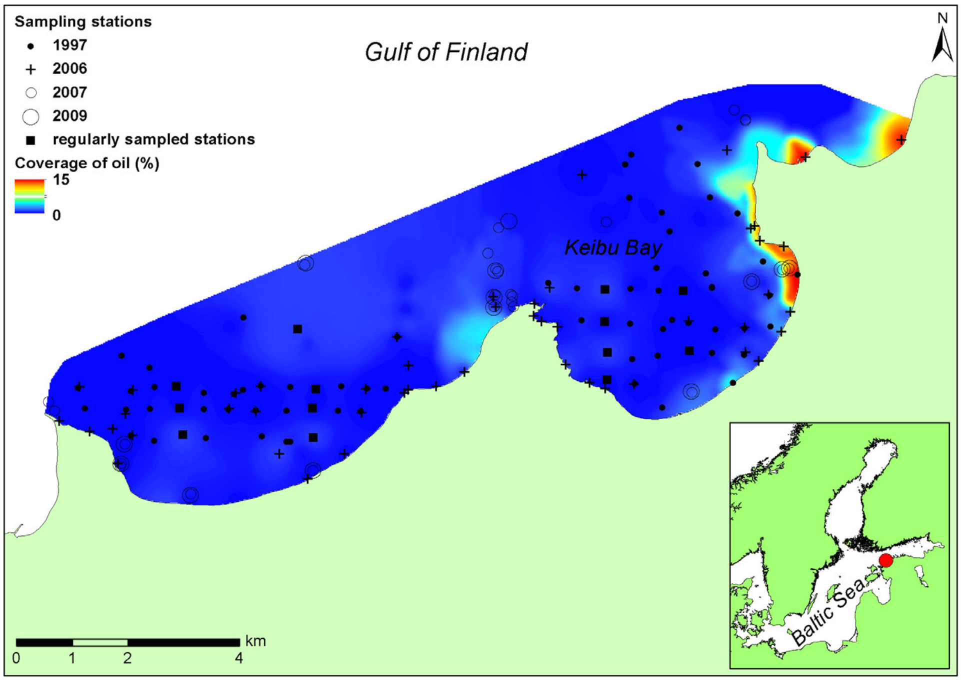

Fig. 1. Study area, biomass sampling stations, and interpolated coverage of oil on the seabed.

Benthos samples were collected in 1997, 2006, 2007, and 2009. The sampling grid differed between years but there were 13 stations that were sampled every year (Fig. 1). An Ekman-type bottom grab sampler (0.02 m^2) was used on soft sediment and a diver-operated metal frame (0.04 m^2) was used to collect samples on hard substrate. All regularly sampled stations were on soft sediment. Samples were sieved through a 0.25 mm mesh and the residuals were placed in plastic bags. The samples were stored deep frozen (–18 °C) until analysis. All invertebrate and macrophyte species were identified in the samples in laboratory except for 1997 when only invertebrates were analysed. Sampling and sample analysis followed the guidelines developed for the HELCOM COMBINE programme (HELCOM, 2008). In all stations the spatial extent of the oil spill, that is percentage coverage, dimensions of oil patches, and thickness of the oil layer, were estimated by a diver in 2006. Besides biomass sampling stations, visual estimates of the oil content were made by a diver in additional locations. Oil was found only in 2006. Data from an unimpacted area near Prangli Island (59.61° N, 25.01° E), Gulf of Finland, were used to compare the temporal dynamics of benthic species. The Prangli site was chosen as a reference site because its environmental conditions (depth, seabed sediment, exposure to waves) were similar to those of the study area.

Except for one set of BEST (Clarke & Warwick, 2001) analyses where data from all stations were included, the analyses were made using data from the regularly sampled stations. Analyses on the data from the regularly sampled stations

(Fig. 1) included only macrozoobenthic taxa. Univariate and multivariate analyses were performed using STATISTICA (StatSoft Inc., 2010) and PRIMER (Anderson et al., 2008), respectively. Inverse distance weighing was used in ArcGIS (ESRI, 2001) software to interpolate the oil coverage on the seabed. ANOSIM (Clarke & Warwick, 2001) was used to test for differences in the structure of benthos between years. The *R* values below 0.2 in ANOSIM analysis were interpreted as negligible separation between groups (Clarke & Gorley, 2001). SIMPER analysis (Clarke & Warwick, 2001) was performed to determine the contribution of individual taxa to the average dissimilarity between the years. The detailed results of SIMPER tests are not shown for brevity. BEST analysis (BVSTEP method) was used to relate the patterns of environmental variables and the structure of benthos (Clarke & Warwick, 2001). DISTLM analysis (Legendre & Anderson, 1999) was run to test how much each environmental variable explained the benthos structure when considered alone (marginal test) and to test the proportion of the explained variation attributable to each environmental variable in the stepwise test. The stepwise test seeks to add a variable only if it improves the explained variation of the model. ANOVA with Tukey's HSD post-hoc test (StatSoft Inc., 2007) was used to assess whether the differences in univariate measures between years were statistically significant. The environmental variables used in BEST, DISTLM, and correlation analyses are shown in Table 1.

Different sets of multivariate analyses were performed on non-transformed, fourth-root-transformed, and presence–absence-transformed abundance and biomass data. Different strength of transformation possibly reveals different aspects of

Table 1. Environmental variables used in analyses

Variable	Explanation	Comments
Oil	Percentage cover of oil on seabed	
Latitude	Geographical latitude	Combined as the variable 'geo' in DISTLM
Longitude	Geographical longitude	
Depth	Depth of water column in sampling station	
Slope	Inclination of the seabed slope	
Aspect	Direction of the inclination of the seabed slope	
Exposure	Exposure to wave action	
Mud	Proportion of mud in seabed sediment	Combined as the variable 'sediment' in DISTLM
Clay	Proportion of mud in seabed sediment	
Fine sand	Proportion of fine sand in seabed sediment	
Medium sand	Proportion of medium sand in seabed sediment	
Coarse sand	Proportion of coarse sand in seabed sediment	
Gravel	Proportion of gravel in seabed sediment	
Pebbles	Proportion of pebbles in seabed sediment	

impact (e.g. Thorne et al., 1999; Anderson et al., 2008). With non-transformed data differences in the abundance or biomass of dominating species override the effects of less abundant species. The fourth-root-transformation diminishes the importance of species with very high abundance or biomass without completely removing differences in abundances or biomasses among species. Presence–absence-transformation retains only the occurrence structure of species without any effect of abundance or biomass values.

Spearman rank correlation was used to test the univariate relationships between environmental (including level of oil pollution) and biological variables. Environmental variables were normalized prior to analyses. The amount of oil that was observed in 2006 was assigned to samples from all years in order to assess whether the correlation between oil and biota is random or causal. If statistically significant relationships between biota and oil were observed only for 2006, then the effects could be attributed to the oil spill. On the other hand, if statistically significant relationships between oil and biota were also found in the other studied years (including the pre-spill year), then the relationships were probably non-causal. Statistically significant relationships between oil and biota in those years when there was no pollution may reflect correlation between natural environmental variables and oil. For the same reason, a special set of BEST analyses was performed. In that set of BEST analyses, the percentage cover of oil on the seabed measured in 2006 was separately assigned to samples from only one year at a time while zero was assigned to all other samples of the other years. If statistically significant relationships between biota and oil were observed only for 2006, then the effects could be attributed to the oil spill. Statistically significant effects in other years probably reflect non-causal relationships. This set of BEST analyses was run on the data that included all sampling stations and both zoo- and phytobenthos. All other analyses were made using only zoobenthos data from the regularly sampled stations.

RESULTS

Distribution of oil

Oil was found only in 2006 and it was unequally distributed in the study area. Oil coverage was high (>5%) only in limited areas near the eastern coastline of the study area (Fig. 1). Only a very low number of sampling stations were located in areas where oil coverage was high.

Differences in the benthic community between years

Although there were many statistically significant results ($p < 0.05$), the separation between years was negligible ($R \leq 0.2$, Table 2). The strongest separation between years was found for the non-transformed abundance structure in 1997 ($R = 0.2$).

Table 2. Differences in the abundance and biomass structure of zoobenthos between years as revealed by ANOSIM. Only data from regularly sampled stations were used. $R > 0.1$ is indicated in bold. Abbreviations: Str. – community structure, Transf. – transformation of community data, abu. – abundance structure of zoobenthos, biom. – biomass structure of zoobenthos, non-tr. – non-transformed data, pr/ab – presence–absence-transformed data, 4root – fourth-root-transformed data

Str.	Transf.	1997 vs other years		2006 vs other years		2007 vs other years		2009 vs other years	
		R	*p*	*R*	*p*	*R*	*p*	*R*	*p*
abu.	non-tr.	**0.200**	0.005	0.084	0.032	−0.005	0.540	0.062	0.034
biom.	non-tr.	0.043	0.297	**0.120**	0.021	−0.060	0.959	0.003	0.427
abu.	4root	**0.151**	0.033	**0.106**	0.019	−0.023	0.722	0.043	0.112
biom.	4root	0.017	0.388	**0.101**	0.022	−0.044	0.908	0.035	0.148
abu.	pr/ab	0.09	0.130	**0.104**	0.027	−0.037	0.840	0.027	0.218

This difference was mainly related to the higher abundance of *Bathyporeia pilosa* and *Macoma balthica* and lower abundance of oligochaetes in 1997 compared to the other years (SIMPER).

Relationships between environmental variables and benthic communities

The proportion of clay and pebbles in the seabed sediment and depth were the most frequently incorporated explanatory variables in the BEST analysis to explain the structure of benthos (Table 3). Oil was selected only once in the model of non-transformed abundance structure of zoobenthos in 1997 (Table 3).

The set of BEST tests performed on the data including all sampling stations but the value of oil coverage (measured in 2006) separately assigned to samples from only one year at a time and zero assigned to all other samples of the other years showed that:

- BEST did not choose oil as an explanatory variable when oil coverage values were assigned to samples from 1997 or 2006;
- when oil coverage values were assigned to samples from 2007, oil was chosen (together with some other variables) as an explanatory variable for the fourth-root-transformed biomass structure and presence–absence structure of zoobenthos and presence–absence structure of phytobenthos;
- when oil coverage values were assigned to samples from 2009, oil was chosen (together with some other variables) as an explanatory variable for the non-transformed and fourth-root-transformed biomass structure and presence–absence structure of both zoobenthos and phytobenthos.

Table 3. Results of the BEST analysis on the relationships between environmental variables and the structure of the benthic community. Only data from regularly sampled stations were used. The best combination of environmental variables is shown for each combination of year, structure, and transformation levels. Only environmental variables that were selected by the stepwise model are shown in this table. The full set of variables is shown in Table 1. Abbreviations: Str. – community structure, Transf. – transformation of community data, abu. – abundance structure of zoobenthos, biom. – biomass structure of zoobenthos, non-tr. – non-transformed data, pr/ab – presence–absence-transformed data, 4root – fourth-root-transformed data. The environmental variables are explained in Table 1

Year	Str.	Transf.	Spearman ρ	p	Oil	Exposure	Slope	Depth	Latitude	Clay	Fine sand	Medium sand	Coarse sand	Pebbles
1997	abu.	non-tr.	0.520	0.119	x				x					x
	biom.	non-tr.	0.403	0.472				x		x		x		
	abu.	4root	0.494	0.217				x	x	x				x
	biom.	4root	0.358	0.460				x		x				
	abu.	pr/ab	0.464	0.289				x		x				x
2006	abu.	non-tr.	0.613	0.001				x		x				x
	biom.	non-tr.	0.672	<0.001				x			x	x		
	abu.	4root	0.676	<0.001				x		x		x		x
	biom.	4root	0.768	<0.001				x				x		x
	abu.	pr/ab	0.672	<0.001			x	x		x	x	x		
2007	abu.	non-tr.	0.496	<0.001				x	x	x	x		x	
	biom.	non-tr.	0.299	0.071						x				x
	abu.	4root	0.530	<0.001						x	x			x
	biom.	4root	0.481	<0.001						x				x
	abu.	pr/ab	0.539	<0.001						x	x			x
2009	abu.	non-tr.	0.507	<0.001			x	x		x	x	x		x
	biom.	non-tr.	0.488	0.002			x	x		x		x		x
	abu.	4root	0.592	<0.001			x	x		x		x		x
	biom.	4root	0.604	<0.001			x	x		x		x		x
	abu.	pr/ab	0.588	<0.001			x	x		x		x		x

DISTLM analysis showed that sediment explained the highest proportion of variance in benthic communities, followed by geographic coordinates and depth (Table 4). Oil was one of the least frequently chosen variables and had statistically significant explanatory power only in the model of non-transformed abundance structure of zoobenthos in 1997.

Based on the results of the SIMPER test, *B. pilosa*, *M. balthica*, and Oligochaeta were the taxa contributing most to the overall dissimilarity between years. ANOVA showed that the abundance of *B. pilosa* and *M. balthica* significantly decreased after 2006 while the abundance of oligochaetes increased. The biomass of *B. pilosa*

Table 4. Proportions of explained variance in community structure attributable to environmental variables as revealed by DISTLM analysis. Only data from regularly sampled stations were used. Proportions in boldface indicate statistically significant values ($p < 0.05$). The marginal test shows the proportion of explanation when each variable was included individually into the models with other variables ignored. Stepwise test seeks to add a variable only if it improves the explained variance of the model. For abbreviations see Table 3. The environmental variables are explained in Table 1

Year	Str.	Transf.	Marginal test							Stepwise test						
			Oil	Exposure	Slope	Aspect	Depth	Geo	Sediment	Oil	Exposure	Slope	Aspect	Depth	Geo	Sediment
1997	abu.	non-tr.	**0.19**	0.04	0.13	0.07	0.12	0.2	0.41	**0.19**	0.05	0.12		0.07		0.37
	biom.	non-tr.	0.02	0.1	0.08	0.1	**0.3**	0.19	0.31		0.08			**0.31**	0.12	0.27
	abu.	4root	0.14	0.05	0.06	0.08	0.16	0.24	0.45						0.19	0.45
	biom.	4root	0.07	0.06	0.07	0.08	0.21	0.18	0.42		0.11			0.21		0.35
	abu.	pr/ab	0.06	0.07	0.05	0.09	0.13	0.19	0.45		0.09			0.12		0.45
2006	abu.	non-tr.	0.04	0.03	**0.08**	0.03	**0.32**	**0.27**	**0.52**				0.03	**0.04**	**0.12**	**0.52**
	biom.	non-tr.	0.06	0.02	0	**0.11**	**0.37**	**0.24**	**0.52**	0.02			0.02	0.04	**0.08**	**0.52**
	abu.	4root	0.05	0	0.06	**0.09**	**0.4**	**0.33**	**0.56**				0.03	**0.06**	**0.12**	**0.56**
	biom.	4root	0.06	0	0.03	**0.16**	**0.46**	**0.34**	**0.63**				**0.04**	**0.06**	**0.06**	**0.63**
	abu.	pr/ab	0.05	0	0.05	**0.18**	**0.39**	**0.31**	**0.59**				**0.07**	0.04	**0.09**	**0.59**
2007	abu.	non-tr.	0.02	0.04	0.05	0.04	**0.15**	**0.2**	**0.31**	0.04	0.02		**0.05**	**0.08**	0.05	**0.31**
	biom.	non-tr.	0.01	0.03	0.02	0.01	0.05	**0.12**	0.11						**0.12**	
	abu.	4root	0.01	**0.08**	0.02	0.04	**0.17**	**0.27**	**0.37**	0.02	**0.07**		0.03	**0.11**		**0.37**
	biom.	4root	0.01	**0.1**	0.01	0.02	**0.14**	**0.25**	**0.25**	0.02	**0.05**		0.02	**0.05**	**0.25**	**0.12**
	abu.	pr/ab	0.02	**0.11**	0.01	0.03	**0.15**	**0.27**	**0.33**		**0.09**		0.02	**0.1**		**0.33**
2009	abu.	non-tr.	0	**0.07**	**0.09**	0.05	**0.1**	**0.18**	**0.36**		**0.03**	**0.05**		0.03	**0.11**	**0.36**
	biom.	non-tr.	0.03	0.07	0.04	0.04	**0.14**	**0.12**	**0.28**	0.02	**0.06**			**0.12**	0.04	**0.28**
	abu.	4root	0	**0.09**	**0.07**	**0.07**	**0.19**	**0.24**	**0.41**		**0.07**	0.02		**0.05**	**0.13**	**0.41**
	biom.	4root	0.02	**0.1**	0.05	**0.06**	**0.22**	**0.23**	**0.35**		**0.1**			**0.15**	0.04	**0.35**
	abu.	pr/ab	0.01	**0.1**	0.05	**0.08**	**0.22**	**0.25**	**0.38**		**0.1**			**0.13**	0.04	**0.38**

decreased over the studied years. The average biomass of *M. balthica* was the lowest and the biomass of oligochaetes the highest in 2007. However, none of the differences in biomasses was statistically significant (Fig. 2). The biomasses of *B. pilosa* and *M. balthica* decreased in the unimpacted reference site near Prangli Island in 2005–2008. Oligochaetes, however, showed different dynamics in the reference site (Fig. 3).

Spearman rank correlation analysis on the data from the regularly sampled stations showed only a few significant correlations between the amount of oil and biotic variables (Table 5). In the year of the oil spill (i.e. 2006), the total abundance

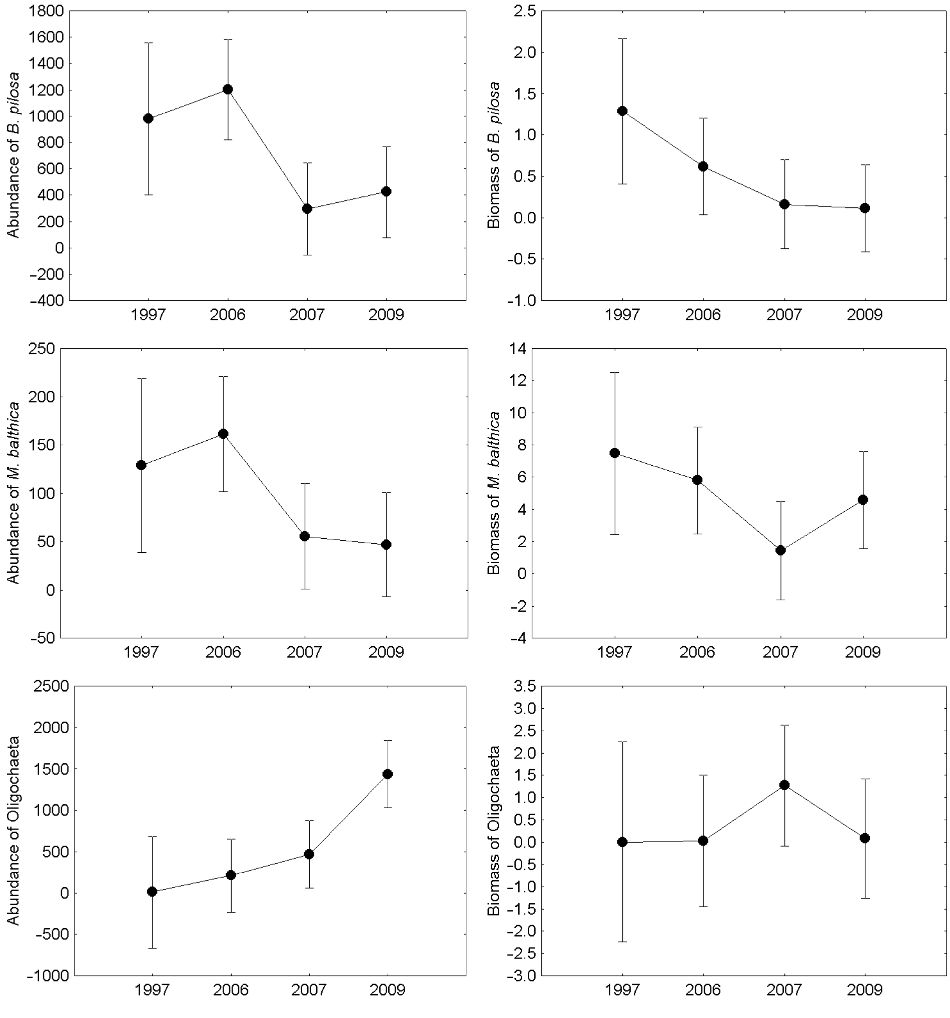

Fig. 2. Changes in the abundance (left column, ind. m^{-2}) and biomass (right column, g m^{-2}) of *Bathyporeia pilosa*, *Macoma balthica*, and Oligochaeta over the studied years. Vertical lines denote 0.95 confidence intervals.

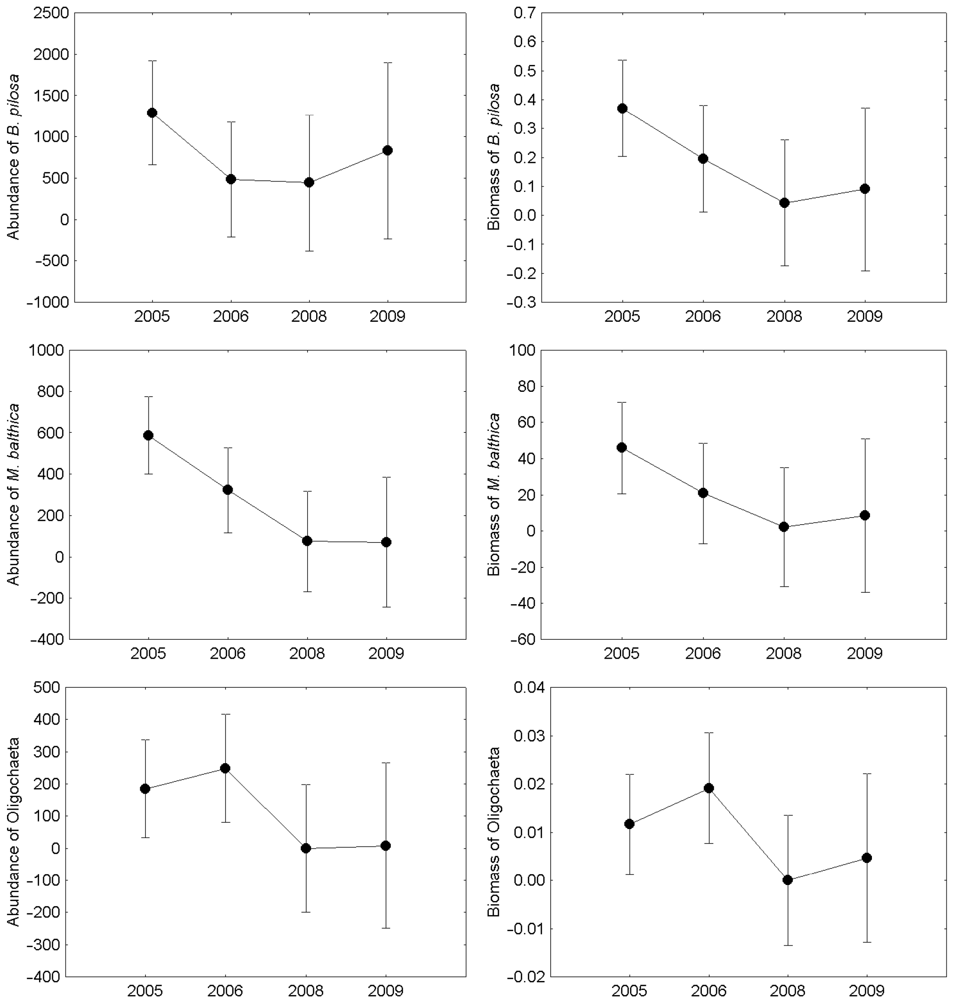

Fig. 3. Changes in the abundance (left column, ind. m^{-2}) and biomass (right column, g m^{-2}) of *Bathyporeia pilosa*, *Macoma balthica*, and Oligochaeta in an unimpacted reference site. Vertical lines denote 0.95 confidence intervals.

of benthic invertebrates and the abundance and biomass of oligochaetes were negatively correlated with the amount of oil. However, when oil coverage values were assigned to samples prior to the oil spill (i.e. reflecting non-causal relationships between oil and biota) we also found a few statistically significant relationships, for example between oil and the total abundance of benthic invertebrates and the abundance of *B. pilosa*. No statistically significant correlations between oil and biota were found in 2007. The abundance of *Hediste diversicolor* was positively correlated with oil in 2009.

Table 5. Spearman rank correlation coefficients between biotic and environmental variables based on the data from regularly sampled stations. Additionally, correlation between the amount of oil and environmental variables is shown. Statistically significant coefficients are shown in boldface. Abbreviations: A tot/B tot – total abundance/biomass of zoobenthos, ZB S – number of zoobenthos species, ZB Shan – Shannon index of zoobenthos, A taxon name/B taxon name – abundance/biomass of a zoobenthos taxon

Variable	Oil	Exposure	Slope	Aspect	Depth	Latitude	Longitude	Clay	Fine sand	Medium sand	Coarse sand	Pebbles
Year: 1997												
A tot	**–0.6**	–0.3	–0.2	0.1	0.1	0.5	**0.6**	–0.1	0.0	0.3	–0.4	0.2
B tot	–0.3	0.2	0.3	–0.2	**0.6**	0.3	0.0	0.4	0.3	0.1	–0.4	–0.3
ZB S	–0.4	–0.4	0.5	0.4	0.1	0.4	0.4	0.1	–0.2	0.3	–0.2	0.2
ZB Shan	–0.3	–0.5	**0.6**	0.5	0.1	0.3	0.4	0.2	–0.2	0.3	–0.2	0.2
A *Bathyporeia pilosa*	**–0.7**	–0.4	–0.1	0.2	–0.1	0.2	0.4	–0.3	0.1	0.3	–0.2	0.1
A *Hediste diversicolor*	–0.4	–0.4	0.1	0.2	–0.2	0.3	0.5	–0.2	0.0	0.2	–0.1	0.2
A *Macoma balthica*	0.0	0.1	0.0	–0.3	0.5	0.4	0.3	**0.6**	0.4	–0.4	–0.2	–0.4
A *Mya arenaria*	–0.5	–0.1	–0.2	–0.2	0.3	0.4	0.4	–0.1	0.3	–0.2	–0.1	–0.2
A *Mytilus trossulus*	0.0	0.1	**0.6**	0.2	0.2	0.0	–0.1	0.4	–0.2	0.2	–0.1	–0.2
A Oligochaeta	0.2	–0.2	0.1	0.5	–0.3	0.2	0.1	–0.2	–0.4	0.2	–0.1	**0.8**
B *Bathyporeia pilosa*	–0.5	–0.3	0.4	0.3	0.1	0.1	0.2	–0.1	–0.1	0.5	–0.2	0.0
B *Hediste diversicolor*	–0.4	–0.4	0.1	0.2	–0.2	0.3	0.5	–0.2	0.0	0.2	–0.1	0.2
B *Macoma balthica*	0.1	0.2	0.1	–0.3	0.5	0.3	0.1	**0.6**	0.5	–0.4	–0.2	–0.4
B *Mya arenaria*	–0.5	–0.1	–0.2	–0.2	0.3	0.4	0.4	–0.1	0.3	–0.2	–0.1	–0.2
B *Mytilus trossulus*	–0.1	0.1	**0.6**	0.2	0.3	0.0	–0.2	0.4	–0.2	0.3	–0.1	–0.2
B Oligochaeta	0.2	–0.2	0.1	0.5	–0.3	0.2	0.1	–0.2	–0.4	0.2	–0.1	**0.8**
Year: 2006												
A tot	**–0.4**	–0.2	–0.2	**–0.4**	**–0.4**	–0.1	0.2	**–0.4**	0.1	0.1	–0.1	–0.2
B tot	–0.2	0.0	0.0	–0.3	**0.5**	**0.5**	**0.5**	**0.5**	**0.7**	**–0.7**	0.0	0.0
ZB S	**–0.4**	0.2	0.0	**–0.6**	**0.5**	**0.4**	**0.4**	0.2	**0.6**	**–0.7**	0.1	0.2
ZB Shan	**–0.4**	0.1	0.1	**–0.4**	**0.6**	**0.6**	**0.5**	0.3	**0.6**	**–0.6**	0.0	0.1
A *Bathyporeia pilosa*	–0.3	–0.3	–0.2	–0.1	**–0.7**	**–0.4**	0.0	**–0.6**	–0.3	**0.5**	–0.2	–0.3
A *Cerastoderma glaucum*	–0.1	0.1	–0.1	–0.2	**0.6**	**0.8**	**0.6**	**0.5**	**0.9**	**–0.8**	–0.1	–0.2
A *Hediste diversicolor*	–0.2	–0.1	–0.3	**–0.5**	0.1	0.3	**0.5**	0.0	**0.4**	–0.2	–0.1	–0.2
A *Macoma balthica*	–0.1	0.1	–0.1	–0.3	**0.7**	**0.7**	**0.5**	**0.6**	**0.9**	**–0.9**	0.0	0.0
A *Mya arenaria*	–0.2	0.2	–0.2	**–0.4**	**0.5**	**0.6**	**0.5**	0.2	**0.7**	**–0.5**	–0.1	–0.2
A Oligochaeta	**–0.5**	0.1	**0.4**	–0.2	0.2	0.2	0.1	–0.2	0.0	–0.2	0.3	**0.4**
B *Bathyporeia pilosa*	–0.2	–0.3	–0.2	0.0	**–0.8**	**–0.6**	–0.1	**–0.6**	**–0.5**	**0.7**	–0.2	–0.3
B *Cerastoderma glaucum*	0.0	–0.1	–0.1	–0.2	**0.6**	**0.8**	**0.6**	**0.6**	**0.8**	**–0.8**	–0.1	–0.2
B *Hediste diversicolor*	–0.3	–0.1	–0.2	**–0.5**	0.1	0.3	**0.5**	0.0	**0.4**	–0.3	–0.1	–0.2
B *Macoma balthica*	–0.1	0.2	0.0	**–0.4**	**0.7**	**0.5**	0.4	**0.6**	**0.7**	**–0.8**	0.1	0.1
B *Mya arenaria*	–0.1	0.2	–0.2	**–0.5**	**0.5**	**0.6**	**0.5**	0.2	**0.7**	**–0.6**	–0.1	–0.2
B Oligochaeta	**–0.4**	0.2	**0.4**	–0.2	0.2	0.1	0.0	–0.2	–0.1	–0.1	0.3	**0.4**

Continued overleaf

Table 5. *Continued*

Variable	Oil	Exposure	Slope	Aspect	Depth	Latitude	Longitude	Clay	Fine sand	Medium sand	Coarse sand	Pebbles
Year: 2007												
A tot	0.2	−0.2	−0.2	0.0	**−0.4**	−0.1	0.1	−0.3	−0.1		−0.1	**0.4**
B tot	0.1	**−0.4**	0.0	0.1	−0.1	0.2	**0.4**	0.2	**0.4**		**−0.6**	0.2
ZB S	0.0	**−0.7**	0.2	**0.4**	**−0.4**	0.0	**0.4**	**0.3**	0.1		**−0.5**	**0.4**
ZB Shan	0.0	**−0.7**	0.2	**0.5**	−0.2	0.1	**0.4**	**0.4**	0.1		**−0.5**	0.2
A *Bathyporeia pilosa*	−0.1	**−0.4**	−0.2	−0.1	**−0.6**	**−0.4**	0.1	−0.3	0.2		−0.3	**0.6**
A *Cerastoderma glaucum*	−0.3	0.0	−0.1	−0.1	0.1	0.2	0.2	−0.1	0.2		−0.1	0.1
A *Hediste diversicolor*	−0.2	−0.1	0.1	0.1	0.0	0.0	0.0	0.1	−0.1		0.0	0.2
A *Macoma balthica*	0.1	−0.3	0.1	0.1	0.3	**0.6**	**0.5**	0.3	**0.4**		**−0.6**	0.1
A *Mya arenaria*	0.0	−0.1	0.0	0.0	0.0	0.0	0.1	−0.1	0.2		−0.2	0.2
A *Mytilus trossulus*	0.0	−0.3	0.1	0.3	−0.3	0.0	0.1	−0.1	−0.2		−0.1	0.3
A Oligochaeta	0.1	0.1	0.0	0.2	−0.2	−0.3	−0.3	−0.2	**−0.5**		**0.6**	0.0
B *Bathyporeia pilosa*	−0.1	**−0.4**	−0.1	−0.1	−0.7	**−0.4**	0.1	−0.3	0.2		−0.3	**0.5**
B *Cerastoderma glaucum*	−0.3	0.0	−0.1	−0.1	0.1	0.2	0.2	−0.1	0.2		−0.1	0.1
B *Hediste diversicolor*	−0.2	−0.1	0.1	0.1	−0.1	0.0	0.0	0.0	−0.1		0.0	0.2
B *Macoma balthica*	0.0	−0.3	0.2	0.2	0.2	**0.5**	**0.4**	**0.3**	0.3		**−0.5**	0.0
B *Mya arenaria*	0.0	−0.1	0.0	0.0	0.0	0.0	0.1	−0.1	0.2		−0.2	0.2
B *Mytilus trossulus*	0.0	−0.3	0.1	0.3	−0.3	0.0	0.1	−0.1	−0.2		−0.1	0.3
B Oligochaeta	0.2	0.0	0.1	0.3	−0.2	−0.3	−0.2	−0.1	−0.3		**0.5**	0.0
Year: 2009												
A tot	0.0	0.1	−0.1	0.0	**−0.5**	**−0.4**	−0.2	**−0.5**	−0.3	0.2	0.2	−0.1
B tot	0.0	−0.1	0.2	0.0	0.3	0.2	0.1	**0.4**	0.2	−0.1	**−0.5**	0.3
ZB S	0.2	**−0.3**	**0.3**	0.3	0.1	0.2	0.2	0.3	0.0	−0.1	−0.2	**0.4**
ZB Shan	0.1	**−0.3**	0.1	0.2	0.1	0.3	0.2	**0.4**	0.2	−0.2	**−0.3**	**0.3**
A *Bathyporeia pilosa*	0.0	−0.2	**−0.4**	−0.1	**−0.5**	**−0.6**	−0.1	**−0.4**	0.2	0.0	−0.1	−0.1
A *Cerastoderma glaucum*	0.1	0.1	−0.3	−0.3	0.2	0.3	0.2	**0.4**	0.2	−0.2	−0.1	0.0
A *Hediste diversicolor*	**0.3**	0.2	0.1	0.0	−0.1	−0.2	**−0.3**	−0.2	−0.3	0.3	0.2	−0.1
A *Macoma balthica*	−0.2	0.2	−0.1	**−0.4**	**0.7**	**0.6**	0.2	**0.5**	**0.4**	**−0.3**	−0.3	0.0
A *Mya arenaria*	0.0	0.0	0.0	0.0	0.1	0.2	0.2	**0.3**	−0.1	−0.2	−0.1	0.3
A *Mytilus trossulus*	0.2	**−0.5**	0.3	**0.5**	−0.1	0.3	0.3	0.2	0.0	−0.2	0.0	**0.5**
A Oligochaeta	0.0	0.2	0.1	−0.1	−0.3	−0.3	−0.3	**−0.5**	**−0.4**	**0.4**	**0.3**	−0.3
B *Bathyporeia pilosa*	0.0	−0.2	**−0.4**	−0.1	**−0.6**	**−0.6**	−0.1	**−0.4**	0.2	0.0	−0.1	−0.1
B *Cerastoderma glaucum*	0.1	0.1	−0.3	−0.3	0.2	0.3	0.2	**0.4**	0.2	−0.2	−0.1	0.0
B *Hediste diversicolor*	0.3	0.1	0.1	0.1	−0.2	−0.2	−0.3	−0.2	−0.3	0.3	0.2	−0.1
B *Macoma balthica*	−0.2	0.2	0.0	−0.3	**0.7**	**0.5**	0.1	**0.5**	**0.4**	−0.3	−0.3	0.1
B *Mya arenaria*	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.3	−0.1	−0.1	−0.1	0.3
B *Mytilus trossulus*	0.2	**−0.5**	0.3	**0.4**	0.0	0.3	0.3	0.2	0.0	−0.2	0.0	**0.5**
B Oligochaeta	−0.2	0.3	0.1	−0.2	−0.1	−0.3	**−0.3**	**−0.4**	−0.3	**0.4**	0.1	−0.2
Oil	1.0	0.0	0.0	0.1	0.1	−0.1	**−0.3**	**0.3**	−0.1	0.0	0.0	−0.1

DISCUSSION

According to previous studies (Kingston et al., 1995; Hawkins et al., 2002; Gómez Gesteira & Dauvin, 2005), soft bottom macrobenthos go through four main phases after an oil spill: (1) a period of rapid mortality of sensitive species (e.g. amphipods), (2) a period of low species diversity and densities, (3) a period of increased abundance of opportunistic species (e.g. oligochaetes and polychaetes), and (4) a period of a decrease in opportunistic species concurrent with recolonization of sensitive species. Our study also indicated some decrease of abundances of sensitive species (*Bathyporeia pilosa*, *Macoma balthica*) and an increase in abundances of opportunistic species (oligochaetes, *Hediste diversicolor*) following the oil spill. However, the overall separation in the community structure between years was very low ($R < 0.3$). When only regularly sampled stations were included in the analysis, the year 1997 showed the highest separation from the other years mainly due to the higher abundances of *B. pilosa* and *M. balthica* and lower abundance of Oligochaeta. The higher separation of 1997 might be attributable to the lack of oil as 1997 was the only pre-pollution year in the analysis. Nevertheless, it is as likely that the observed differences were due to the higher temporal separation of 1997, that is years closer in time are expected to be more similar than distant years.

Oil pollution is known to have an unfavourable effect on amphipods (Bonsdorff, 1981; Jewett et al., 1999; Ocon et al., 2008) but also the bivalve *M. balthica* (Stekoll et al., 1980), and so their decreasing abundances may be related to the oil spill. The abundances of *B. pilosa* and *M. balthica* were high in the year of the pollution but had significantly dropped by 2007. This pattern may be related to the timing of sampling. Namely, the sampling was carried out about three months after the pollution in spring 2006 when the effect of oil might had not reached its maximum impact. However, the abundance and biomass of *M. balthica* and the biomass of *B. pilosa* also decreased in the unimpacted reference site during 2005–2008, indicating that the decrease might have been due to natural interannual variability.

Topographical features and substrate type were the most important environmental factors that described the structure of benthos. According to BEST analysis, the percentage cover of oil was an important variable explaining the non-tansformed abundance structure of zoobenthos in 1997. As there was no pollution in 1997, the statistical effect of oil seems to reflect correlations between oil and several other environmental variables. Such analyses demonstrate the importance of statistical tests that assess whether the observed relationships reflect possible causal relations or collinearity between independent variables or even random effects. In the set of BEST tests where the oil coverage (measured in 2006) was separately assigned to samples from only one year at a time while zero was assigned to all the other samples from the other years, the number of tests that chose oil as an important explanatory variable of biota was the highest in 2009. The emergence of the effects of the oil pollution as late as in 2009 is not likely, as the strongest effect of oil is expected to appear immediately after pollution (Bonsdorff, 1983). Instead, the results indicate that the distribution of oil in 2006 was related to several natural environmental variables, which, in turn, caused statistically significant correlations between oil and biota to emerge. Field observations showed that oil was more likely to accumulate close to peninsulas, that is in areas characterized by steep coastal slopes and high exposure to waves.

Oil was the second least frequently chosen variable in DISTLM analysis. When oil was chosen by the model, it explained 2–19% of the overall variability. However, the relationship between oil and biota was not causal as these models represented situations when oil coverage values were artificially assigned to years prior to the oil spill. As there was no pollution prior to the current oil spill, this result indicates either correlation of oil with some natural environmental variables or a random chance. Furthermore, this proves the validity of our approach to statistical testing of the effect of oil in each year not only the year of pollution. Such methodology enables some implication of the causality in the correlations between oil and biota: significant correlations only in the year of pollution refer to possible causal relationships while significant correlations in other years (namely those before pollution) hint a collinearity of oil and natural environmental variables or a random chance.

Similarly, univariate rank correlation analysis did not reveal explicit effects of oil. The total abundance of benthic macroinvertebrates was negatively correlated with oil in 1997. Additionally, the abundance of the sensitive amphipod *B. pilosa* was negatively correlated with oil in 1997. Moreover, the total abundance of zoobenthos was negatively correlated with oil in 2006. This indicates that oil accumulated in areas that had naturally lower density of macroinvertebrates. The abundance of *H. diversicolor* was positively correlated with oil in 2009, possibly indicating the facilitative effect of oil on detrivores (Peterson, 2001).

In addition to the characteristics of the pollution load, timing, and the environment, the sampling design made distinguishing the effect of oil difficult. The amount of oil was very low or non-existent in most of the study area and the sampling grid insufficiently covered the areas with a high oil content. As the patterns of oil pollution are unpredictable, it is impossible to apply a proper BACI design (see Underwood, 1992), which leads to difficulties in distinguishing between the effects of natural environmental factors and oil on biota. Regardless of the shortcomings of the design, if there had been major bay-scale changes in the communities due to oil, the statistical analyses would have shown them. Some patterns such as the decrease in the abundance of *B. pilosa* and *M. balthica* may be related to the oil spill, but without clear experimental evidence it would be speculative to attribute this pattern solely to oil. Regardless of the lack of evidence of strong acute effects, it cannot be concluded that the oil spill has had no local effect on benthos at all, especially in sites with oil coverage higher than 10%. Those sites correspond to the situation where we lacked a proper BACI design. Moreover, in this study we most likely missed long-term sublethal effects as such evidence is very difficult to demonstrate from changes in the abundance and biomass. For example, the

Exxon Valdez oil spill in Alaska, USA, showed surprisingly long-lasting sub-lethal effects on the ecosystem (Peterson et al., 2003).

The statistical analyses did not show explicit effects of oil on benthos. The reasons may be related to the small amount of spilled oil together with the peculiarities of the area, that is a high wave exposure and sandy seabed sediments. Oil was probably quickly washed ashore in the exposed environments of the study area, while hydrodynamic activity removed toxic compounds and provided oxygen. Similarly to the current study, Mustonen & Tulkki (1972) could not elucidate the effect of more than 100 tonnes of spilled oil on benthos in the hydrodynamically active northern Baltic Proper. Bonsdorff (1984) found that the recovery from a small-scale oil spill in moderately exposed rock pools took place within weeks to about a month. Exposed sandy sediments host low-diversity communities that cope with high physical disturbances. The effects of oil pollution of the same magnitude might have had much stronger effects in a sheltered bay with diverse and abundant vegetation as shown by Notini (1978).

It has been shown that the effect of oil is stronger when the pollution coincides with an annual spat of some key species (Lindén, 1976; Notini, 1978). The spill was detected in January, which is long before the reproduction season of zoobenthos. This allowed most of the toxic compounds in the oil to dilute and evaporate before the spat. Pollution in spring or summer might have resulted in more severe effects. Lindén (1976) showed that juveniles of *Gammarus oceanicus* are hundreds of times more sensitive to oil than adults.

To conclude, our results showed absence of clear effects of the oil spill on benthos. The decrease in the abundance of *Bathyporeia pilosa* and *Macoma balthica* may be related to the oil, but without a proper BACI design with several control locations it would be speculative to attribute this pattern solely to oil. Our study clearly showed that in the case of accidental environmental impacts such as oil pollution, it is impossible to apply a proper setup of a BACI design, which leads to difficulties in distinguishing between the effects of natural environmental factors and oil on the biota. The study also advocates for needs of alternative methodologies in order to effectively assess the impacts of accidental anthropogenic disturbances on benthic communities.

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Naftareostuse ökoloogilise mõju hindamine riimveelistele põhjakooslustele: juhtumiuuring Läänemere kirdeosas

Kristjan Herkül ja Jonne Kotta

Seoseid abiootiliste keskkonnamuutujate ja makrobentose struktuuri vahel uuriti Soome lahe edelaosa madalates lahtedes, kus 2006. aastal oli naftareostus. Põhjaelustiku struktuuri kirjeldasid kõige paremini merepõhja sette iseloom, sügavus ja avatus lainetusele. Vaatamata kogutud proovide suurele hulgale, ei olnud statistiliste analüüside tulemusel võimalik selgelt eristada nafta mõju põhjaelustikule. Põlvikvähi (*Bathyporeia pilosa*) ja balti lamekarbi (*Macoma balthica*) arvukuse vähenemine võib olla seotud naftareostusega, kuid ilma põhjaliku BACI (*before-after-control-impact* – "enne-pärast-kontroll-mõju") disainita on looduslikku muutlikkust ning naftast tingitud muutusi uuritud kooslustes võimatu eristada. Kuna õnnetusjuhtumite toimumise aeg ja koht on ette teadmata, siis klassikalise BACI disaini rakendamine naftareostuse mõjude uurimiseks on võimatu, mis teeb loodusliku muutlikkuse ning õnnetusest tingitud mõjude eristamise äärmiselt raskeks. Tulemus näitab, et õnnetusjuhtumite mõju hindamiseks on vaja uusi metoodikaid.