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Seasonal and geographical biomarker variability

D 3.7

WP3: Determination of oil and dispersant impacts on biota using effect-based tools and ecological risk assessment

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Executive Summary

Biomarkers measured in organisms indicate exposure and effects of chemical contaminants on various functions at different biological levels. However, apart from showing species-specific differences, many of them are also sensitive to other environmental stressors such as temperature, salinity, and dissolved oxygen levels. Natural seasonal fluctuations in physical conditions and food availability also regulate intrinsic factors including bioenergetic status and reproductive stage, which in turn regulate baseline levels of some biomarkers. Therefore, understanding of the natural variability and baselines at a local or regional scale is essential in using biomarkers in monitoring programmes; without this knowledge the risk of incorrect interpretations is high. In the high-latitude Baltic Sea, variations in environmental conditions is significant during the year. In addition, permanent salinity gradients prevail in the area, affecting the physiology of organisms in different parts of the sea. Thus, information is needed on biomarker baseline levels in mussels (*Mytilus* spp.) in different sea areas. This report summarises the ranges of variability in selected biomarkers investigated during the GRACE project including information on background tissue levels of polycyclic aromatic compounds (PAH).

1. Rationale

Mussels of the genus *Mytilus* are commonly used biomonitoring organisms worldwide (Beyer et al., 2017). Understanding of the natural variability and baseline values at a regional scale is essential for a proper identification of risks as well as to design and implement efficient monitoring programmes and surveillance strategies. The above is true to all types of pollution monitoring and assessment, including marine oil spills as well as the impacts of response actions. Confounding physical factors related to latitude (temperature and photoperiod) as well as biological determinants (gender, age) causing short and long-term temporal variability must be considered in biological effects assessment using biomarkers (Leiniö and Lehtonen, 2005; Garmendia et al., 2010; Izagirre et al., 2014). Thus, reliable baseline data is an issue of major importance for implementing the assessment of adverse biological effects, also those caused by oil spills and oil spill response. In this report, the focus will be on the brackish-water Baltic Sea.

2. Special features of the Baltic Sea

Compared to other European sea areas the semi-enclosed Baltic Sea exhibits numerous important abiotic and biotic characteristics that make it exceedingly different in regard to various processes. Important specific physical characteristics include: (1) steep salinity gradients (from ca. 20 psu to 8 psu) in the south-western part and a further reduction from the southern to the northern parts (from 7 psu in the Baltic Proper to ca. 2 psu in the eastern Gulf of Finland and Bothnian Bay), (2) large seasonal variability in temperature, (3) ice cover in winter (mainly in the northern part), and (4) temporal oxygen deficiency not only in the deeper basins but also on many coastal areas. As a result of these specific hydrographical and climatic conditions, species diversity in the Baltic Sea is reduced compared, e.g., to the North Sea). In this respect, salinity is a key factor, since many marine species reach their tolerance limit at salinities below 12 psu. Owing to the variability in the composition of local biota in the north-south axis of the Baltic, the same species may not necessarily be applicable as bioindicators for the entire Baltic Sea area. Moreover, species may react differently towards contaminants than in a high-saline environment (Lehtonen and Schiedek, 2006).

The mussel *Mytilus* spp. is present in the Baltic Sea as *M. trossulus* and *M. edulis*, as well as their hybrids. It is evident that these closely related species may have differences in regard to biological responses to various pollutants; however, basing on current knowledge and lack of detailed comparisons from the Baltic Sea, for practical reasons the possible dissimilarities are regarded as of minor importance. Since the low-salinity tolerance range of *Mytilus* spp. is around 4 psu they do not occur in the most low-saline northern parts of the Baltic Sea (Bothnian Bay and Eastern Gulf of Finland) or, due to the virtually non-existent tide, close to the river mouths of major rivers. Therefore, for these areas other biomonitoring species have to be considered.

Due to the highly varying environmental conditions during the season (especially in the northern part of the Baltic Sea), marked seasonal variability can be seen in most of the biomarkers (Leiniö and Lehtonen 2005). This variability has to be taken carefully into account prior to make interpretations of the measured values in each monitoring parameter, and in fact more information is still needed on the variability.

3. Sampling of mussels for the establishment of baselines

In GRACE, mussel sampling in the Baltic Sea consisted of three seasonal samplings (spring, summer, and autumn) carried out in presumably less contaminated sites (considered as reference) in the northern (Tvärminne, Gulf of Finland) and southern (Kiel Bight) parts of the sea (Fig. 1). The results and experiences gained from this activity complement markedly to previous data on baseline levels and seasonal variability in a selection of biomarkers measured in mussels in different parts of the Baltic Sea (Leiniö and Lehtonen 2005, Baršienė et al. 2006, Kopecka et al. 2006, Schiedek et al. 2006).

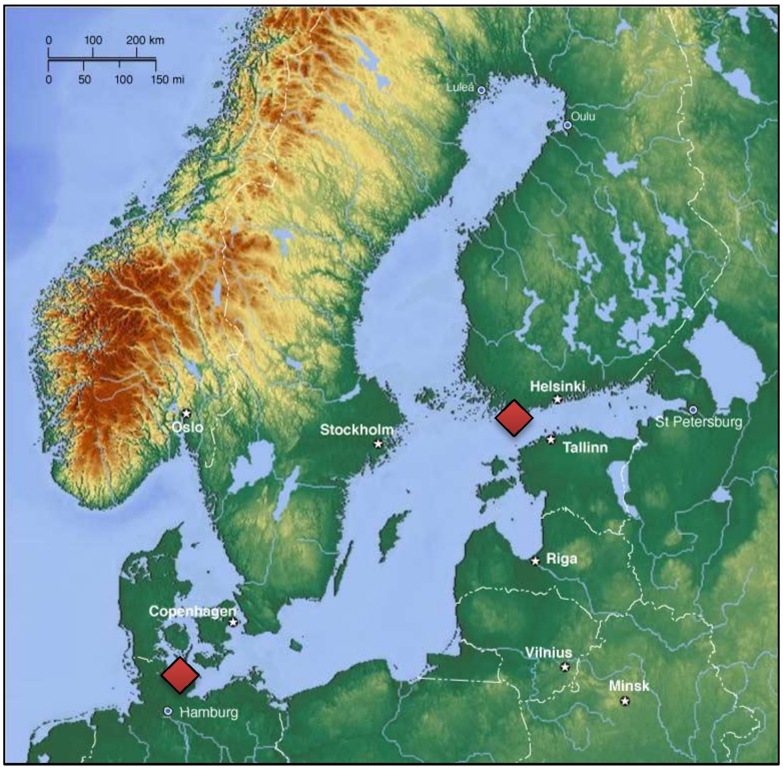

Fig. 1. The Baltic Sea, with the seasonal sampling sites of mussels (red diamonds). Map from the "One World - The Nations Online Project" (http://www.nationsonline.org)

Due to the lack of tide in the Baltic Sea, mussels usually need to be collected by SCUBA divers. Immediately after the dive, mussels were sorted and about 100 mussels of size 2.5-4.0 cm were placed to thermo-insulated containers with water from the collection site. Mussels were taken to laboratory, kept in ambient water and temperature until next day when the dissection took place.

Table 1. Details of mussel sampling.

	FINLAND			GERMANY			
	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer
Sampling area	Tvärminne Brännskär, Hanko			Kieler Förde, Kiel/Holtenau			
Date	7.7.2016	26.10.2016	4.4.2017	16.8.2016	16.11.2016	14.3.2017	18.8.2017
Depth m	7-8	7-8	7-8	0.2	0.2	0.2	0.2
Temperature °C	15	10	4	17	6	5	18

4. Results

4.1. Background levels in biomarkers

Seasonal ranges of the measured biomarkers at the two Baltic Sea sampling sites are given in Table 2.

Table 2. Seasonal ranges of biomarkers at Tvärminne (Finland) and Kiel (Germany) in the Baltic Sea. AChE acetylcholinesterase; GST glutathione S-transferase; CAT catalase; GR glutathione reductase; LPO lipid peroxidation; Vv_{LYS}, volume density of digestive cel lysosomes; Vv_{LPF}, volume density of lipofuscins; VvNL, vlume density fo neutral lipids; VvBAS, volume density of basophilic cells; CTD, connective-to-diverticula ratio; ADG connective tissue adipogranular cells; ^{sp}, spring; ^{a}, autumn; ^{su}, summer.

Biomarker / tissue	Units	Tvärminne	Kiel
AChE / gills	nmol min^{-1} mg protein^{-1}	2.0^{sp}-8.0^{a}	3.0^{sp}-7.0^{su}
GST / digestive gland	nmol min^{-1} mg protein^{-1}	71.0^{a}-136.0^{su}	91.0^{a}-134.0^{sp}
GST / gills	nmol min^{-1} mg protein^{-1}	182.0^{sp}-380.0^{a}	159.0^{a}-232.0^{su}
CAT / digestive gland	µmol min^{-1} mg protein^{-1}	31.0^{a}-50.0^{su}	35.0^{su}-70.0^{sp}
CAT / gills	µmol min^{-1} mg protein^{-1}	14.0^{su}-23.0^{a}	14.0^{a}-18.0^{su}
GR / gills	nmol min^{-1} mg protein^{-1}	10.0^{sp}-13.0^{a}	8.0^{a}-19.0^{su}
LPO / digestive gland	nmol mg wet wt	101.0^{sp}-154.0^{a}	97.0^{sp}-145.0^{a}
Vv_{LYS} / digestive gland	µm^{3}/ µm^{3}	<0.0028	<0.0065
Vv_{LPF} / digestive gland	µm^{3}/ µm^{3}	0.0710^{su}-1.000^{sp}	0.0508^{su}-0.8297^{a}
Vv_{NL} / digestive gland	µm^{3}/ µm^{3}	0.0480^{a}-0.2196^{su}	0.0664^{su}-0.1012^{a}
Vv_{BAS} / digestive gland	µm^{3}/ µm^{3}	<0.1030	<0.1309
CTD ratio / dig. gland	-	0.1537^{sp}-0.4134^{su}	0.3071^{su}-0.4757^{sp}
Atrophy Index / dig. gland	-	1.575^{sp}-2.575^{a}	1.653^{au}-2.250^{su}
ADG index / mantle	-	0.525^{sp}-1.425^{au}	0.300^{ap}-1.600^{au}
Gonad Index / mantle	-	3.4^{sp}-4.2^{su}	1.4^{su}-3.4^{sp}

4.2. Background levels of PAH

Background levels of 24 polycyclic aromatic compounds (PAHs) measured in mussels collected in spring, summer and autumn at the sampling sites in Finland and Germany showed low values of individual PAHs in tissues, many of them being below the detection limit of the method used (0.5 or 1.0 ng g^{-1} wet wt, depending on substance). The highest values (ng g^{-1} wet wt) of the substances above the detection limit are given below: 1-methylnaphthene: 1.2, 2-methylnaphthene: 2.5, 9-methylphenanthrene: 1.3, benzo(*a*)antracene: 2.1, benzo(*b*)fluoranthene: 2.5, phenanthrene: 2.0, fluoranthene: 7.1, and triphenylene 5.4. One notable exception has to be mentioned: in spring 2017, an extremely high tissue level (1500 ng g^{-1} wet wt) of naphthalene was measured at the Finnish site, apparently caused by some leakage from a yet unknown source. During all the other sampling campaigns the level of this substance was close or below the detection level.

Apart from the anomalous value for naphthalene the levels of almost all the PAHs measured above the detection level were in general slightly higher at the German site compared to the Finnish site. Compared to a recent mussel caging monitoring exercise carried out at five presumably contaminated sites along the coast of Finland, the values measured are considerably lower and can be taken to represent a clean site in this part of the Baltic Sea.

5. Concluding remarks

Seasonal baseline levels defined here for a battery of biomarkers in mussels in different parts of the Baltic Sea serve as vital background information in case of pollution incidents, including oil spills and remediation actions taking place in the area. Both temporal and spatial variability in most of them is significant, underlining the importance of local/regional baseline values for correct interpretations. Concentrations of PAH in the tissues measured from the same batches of mussels confirm that, at least concerning these compounds, the measured biomarker levels represent relatively clean areas of the Baltic Sea. Overall, the results obtained in GRACE bring important new information to complement the previous data collected from the area on much-needed baseline levels of biomarkers.

6. Acknowledgement

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