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Biomarker responses and accumulation of hazardous substances in mussels (*Mytilus trossulus*) transplanted along a pollution gradient close to an oil terminal in the Gulf of Finland (Baltic Sea)

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ABSTRACT

Baltic Sea blue mussels (*Mytilus trossulus*) were used as sentinel organisms to detect the biological effects of chemical contamination in the low salinity environment. Mussels naturally adapted to a salinity of ca. 6.0 PSU were caged for 30 days at four sites along an assumed pollution gradient (salinity ca. 4.5 PSU) in the vicinity of Finland's largest oil refinery and harbor Kilpilahti in the Gulf of Finland. Tissue concentrations and accumulation rates of especially organic contaminants (PAHs, PCBs and organotins) were clearly elevated at the innermost coastal stations near the harbor area. Biological effects of contaminant exposure on caged mussels were evaluated by measuring a suite of biomarkers including catalase, glutathione S-transferase, superoxide dismutase, glutathione reductase, lipid peroxidation, acetylcholinesterase activity and lysosomal membrane stability. Mussels transplanted near the harbor area were able to elevate their antioxidant defense in response to environmental contamination. Reduced morphometric condition index and soft tissue growth rate together with increased lipid peroxidation and low lysosomal membrane stability were also observed at the most contaminated site. The results suggest that caging of *M. trossulus* for four weeks at lower salinity is a feasible method for the detection of environmental pollution also in low salinity areas of the Baltic Sea.
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1. Introduction

Coastal and estuarine ecosystems are subjected to many stressors, including salinity fluctuations and anthropogenic disturbance. In the Baltic Sea, the influence of these factors is even more pronounced due to a large, highly urbanized and agricultural catchment area and very low water turnover time (HELCOM, 2010). Marked increases in oil tanker traffic, offshore oil production activities and the number of oil terminals possess a growing threat of accidents and severe oil spillages. Also, detection of the general deterioration of water quality caused by the lesser noticed, constant release of oil related compounds into the environment requires monitoring activities. In the low-diversity northern Baltic Sea relatively few species are suitable for the biomonitoring of environmental pollution. The blue mussel (*Mytilus* spp.) is a commonly used monitoring species worldwide and abundantly present also in the Baltic Sea. However, its occurrence is limited by permanently low salinity (<4.5 PSU) prevailing in the central-eastern part of the Gulf of Finland and in the Gulf of Bothnia (Bothnian Bay) (e.g. Westerbom et al., 2002).

In this study, mussels were caged on the verge of their natural distribution at the salinity of ca. 4.5 along a suspected pollution gradient close to a large oil terminal in the Gulf of Finland. The Kilpilahti harbor is in the close proximity of the Fortum Oil refinery, one of the largest in Scandinavia, producing over 200,000 barrels a day with the annual capacity of 11 million tons. The area under study is constantly exposed to heavy maritime traffic with large tankers carrying crude oil from Russia and the North Sea to the Kilpilahti harbor, with the refined oil products being shipped to Russia and many other destinations. Apart from these sources of potential pollution, local industry, municipal waste waters and agriculture drain-off contribute strongly to the contaminant status of this coastal region.

Potential oil pollution being the main issue in this area of polycyclic aromatic hydrocarbons (PAHs) is of special interest as PAHs are considered among the most toxic components of oil related compounds. Because of all the other potential pollution sources, tissue concentrations of polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), organotins and selected trace metals were also determined. According to a recent assessment of hazardous substances in the Baltic Sea carried out by HELCOM these contaminants mentioned above show the highest concentrations in relation to the threshold levels for biological effects (HELCOM, 2010).

Biomarkers are increasingly used in the monitoring of biological effects of contaminants in the marine environment see (e.g. ICES, 2011). Among these, antioxidant enzyme responses in mussels have been successfully used in many studies to reveal chemical exposure

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and to assess biological effects of contaminants in impacted coastal areas (Viarengo and Canesi, 1991; Orbea and Cajaraville, 2006; Lima et al., 2007; Vidal-Linan et al., 2010). Exposure to hazardous chemical substances stimulates the formation of reactive oxygen species (ROS) ensued by oxidative damage to membrane lipids, proteins and DNA (Livingstone, 2001). Cells are protected against the deleterious effects of oxyradical generation by maintaining ROS at low levels through the action of several antioxidant enzymes (Livingstone et al., 1990; Valavanidis et al., 2006; Kaloyianni et al., 2009). The antioxidant enzyme system operates as a complex battery of activation and inhibition responses depending on the environmental conditions and the contamination degree. In general, it is suggested to use a multibiomarker approach to investigate the enzymatic responses in order to clarify situations where most of the used biomarkers are significantly altered either positively or negatively, or to detect possible "bell-shape" responses (Frenzilli et al., 2004; Orbea and Cajaraville, 2006).

The following oxidative stress biomarkers were selected to investigate the antioxidant defense responses in mussels: glutathione S-transferase (GST; EC 2.5.1.18), which functions in detoxification and antioxidant defense; catalase (CAT; EC 1.11.1.6), glutathione reductase (GR; EC 1.8.1.7) and superoxide dismutase (SOD; EC 1.15.1.1), that are part of a major enzymatic defense system to remove ROS; and lipid peroxidation (LPO), which indicates the damage to cellular membrane lipids caused by ROS. In addition to the oxidative stress biomarkers, acetylcholinesterase inhibition (AchE; EC 3.1.1.7) revealing neurotoxic effects as well as general stress and lysosomal membrane stability (LMS) detecting cellular damage and effects on the immune system were measured from the caged mussels.

The main aim of this study was to investigate the potential of using caged *Mytilus trossulus* as a bioindicator species in the northern Baltic Sea. As stated above, the constantly-low salinity (<5) is a specific feature of the study area; by caging mussels along an assumed contamination gradient we set out to study the response levels in selected biomarkers and the accumulation of some important contaminants under these environmental conditions.

2. Materials and methods

2.1. Collection of experimental mussels and the caging procedure

In a recent study Väinölä and Strelkov, 2011 demonstrated that according to a common genetic marker (Gpi), *Mytilus* populations occupying Baltic Sea coasts form southern Sweden up to the Bothnian Sea and the Gulf of Finland are pure *M. trossulus*. However, when these mussels are compared to *M. trossulus* specimens inhabiting other sea areas, a special historical influence of *Mytilus edulis* can be seen present throughout the Baltic Sea basin. The authors concluded that the Baltic *Mytilus* represents a single, locally uniform and geographically homogenous gene pool, and recommended that "Baltic *M. trossulus*" should be used as the name of the species.

Adult mussels of similar size (2.2–3.0 cm shell length) were used in the caging experiment. Mussels were collected on September 6, 2007 by scuba diving in Hanko, Western Gulf of Finland, a site considered (by Baltic Sea standards) relatively clean from anthropogenic pollution (Fig. 1). Salinity at the sampling site is ca. 6.0. The mussels were immediately transported to the laboratory in thermo-insulated water buckets and placed in aerated tanks filled with water from the collection site until deployment in cages three days later. For the determination of Day 0 (prior to caging) biomarker levels and contaminant concentrations a group of mussels was dissected in the laboratory directly after arrival from the collection site and the tissues were frozen at −80 °C.

Caging of the mussels took place aboard r/v Aranda and the caging period was between September 9 and October 9, 2007 (30 d). Four cages were deployed according to an assumed pollution gradient from the Kilpilahti oil harbor towards the outer archipelago (Fig. 1).

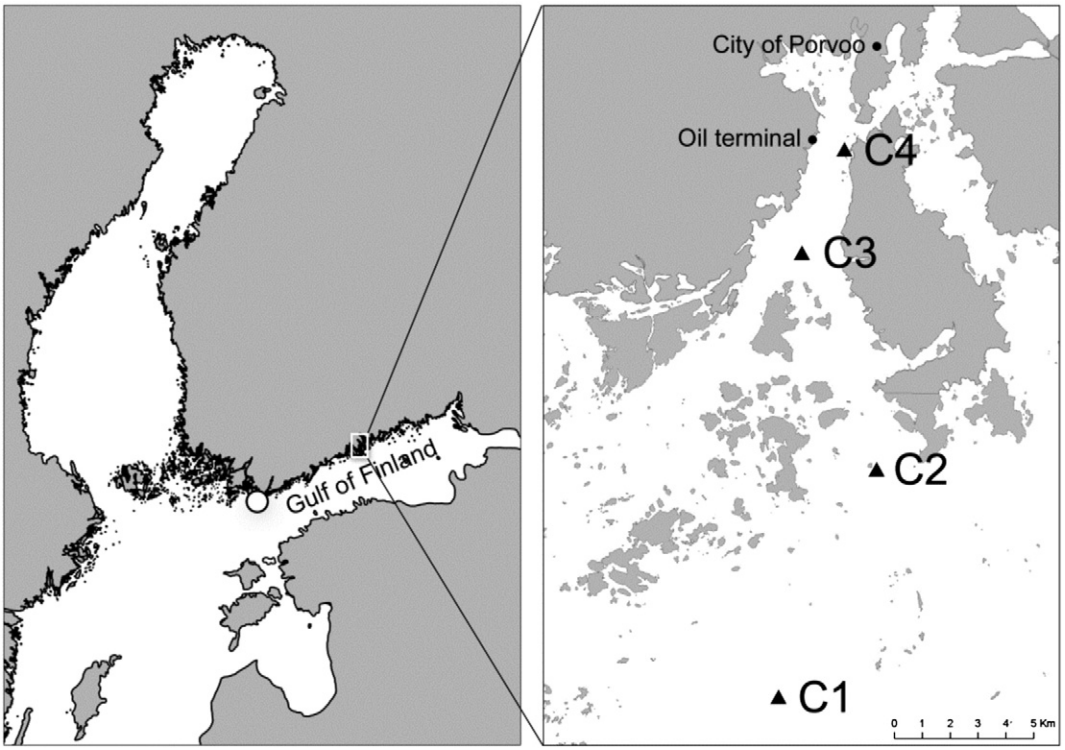

Fig. 1. The study area. The circle on the left map indicates the collection site of the mussels and the square shows the study area in the Gulf of Finland. The map on the right shows the locations of the mussel cages (C1 to C4).

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