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Enzymatic and cellular responses in relation to body burden of PAHs in bivalve molluscs: A case study with chronic levels of North Sea and Barents Sea dispersed oil

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ABSTRACT

Mytilus edulis and *Chlamys islandica* were exposed to nominal dispersed crude oil concentrations in the range 0.015–0.25 mg/l for one month. Five biomarkers (enzymatic and cellular responses) were analysed together with bioaccumulation of PAHs at the end of exposure. In both species, PAH tissue residues reflected the exposure concentration measured in the water and lipophylicity determined the bioaccumulation levels. Oil caused biomarker responses in both species but more significant alterations in exposed *C. islandica* were observed. The relationships between exposure levels and enzymatic responses were apparently complex. The integrated biomarker response related against the exposure levels was U-shaped in both species and no correlation with total PAH body burden was found. For the monitoring of chronic offshore discharges, dose- and time-related events should be evaluated in the selection of biomarkers to apply. From this study, cellular damages appear more fitted than enzymatic responses, transient and more complex to interpret.

1. Introduction

An ongoing concern related to the development of offshore oil and gas operations in the North Sea is that long-term exposure to operational drilling and waste water may cause detrimental effects to marine organisms. Currently, the amount of produced water, the main waste water effluent, is increasing because of aging of the fields and prospects predict a continuation of the increase in the coming years. There are reasons for environmental concern regarding a number of contaminants discharged in the produced water effluents of the North Sea. These effluents are currently being monitored for contaminants input and biological effects (Hylland et al., 2008).

Offshore activities are also moving towards more remote sub-Arctic marine environments with unique aspects like the marked seasonal variation in light, colder temperature and the presence of sea ice at some times of the year. These cold environments host a wide variety of marine life, including some species which are important economical resources. In the Barents Sea, there is a strong official requirement to prevent long term environmental impacts. Presently, few data are available on chronic toxic effects of offshore discharges in this relatively cold marine environment and a zero discharge policy is applied in a conservative way. For the Barents Sea ecosystem, deficiency in sub-sea pipelines can lead to release of undetected chronic level of oil at times but the major threat remains related to massive discharges of oil contaminants in the course of production, processing or transport.

A relevant issue to address is whether the monitoring tools and risk assessment that currently prevail in the temperate regions can be applied to determine the sensitivity and predict effects on Arctic marine organisms. This can be appraised by understanding how biological responses are modified under the conditions where these organisms are living. In the past, monitoring was based mainly on analyses of chemicals like polycyclic aromatic hydrocarbons (PAH) and alkylphenols, two classes of toxic pollutants found in waste water. Determining when contamination has resulted in adverse effects in the natural environment requires a combination of chemical and biological measurements. The need to use integrated measurements of actual effects on biota has been strengthened during the last decades. Environmental monitoring programmes including both chemical analyses of seawater for contaminant levels and the measurements of a battery of so-called biomarkers are presently recommended (Cajaraville et al., 2000; Galloway et al., 2004; Moore et al., 2004). Although the use of biomarkers for ecological risk assessment is still debated (Forbes et al., 2006), biomarkers are presently becoming part of the health assessment and management of aquatic ecosystems in addition to the more traditional water chemical analyses (Hagger et al., 2008; Allan et al., 2006). Discussions within ICES/OSPAR are also

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ongoing to determine protocols of application and select which biological markers to use for different biota (ICES, 2008a). These recommendations serve as a good basis on how water column monitoring surveys around platforms are applied (Gorbi et al., 2008; Hylland et al., 2008) and more generally for the environmental management of seawater where offshore industries are present.

In the Biosea I JIP program, a biomarker-based approach has been chosen. Laboratory-controlled experiments were performed to implement and evaluate a suite of relatively well established biomarkers on several marine organisms for their primary use in offshore oil and gas monitoring programmes in the North Sea and in the Barents Sea. Long-term exposures to several concentrations of dispersed crude oil were conducted with different species. Fish and invertebrates (echinoderms, crustaceans and bivalve molluscs) were selected. Here, we present the data obtained with molluscs.

Although bivalve molluscs are more associated with benthos, they are extensively used in biomonitoring programmes and for example deployed in caging experiments around platforms to monitor both chemical concentrations and biological responses (Hylland et al., 2008). Their sessile, filter feeding lifestyle and low enzymatic degradation rate render them capable of retaining relatively higher levels of organic molecules compared to other organisms (Meador et al., 1995).

Two mollusc species were used in this study. For the North Sea, we used the blue mussel *Mytilus edulis* and the Icelandic scallop *Chlamys islandica* was selected for the Barents Sea. Blue mussels have been largely deployed for monitoring contaminant concentrations in the water as well as to evaluate a number of selected biomarkers in the laboratory or in real field conditions (Bocquene et al., 2004; Bodin et al., 2004; Orbea et al., 2002; Porte et al., 2001; Regoli et al., 2002; Aarab et al., 2004). Ecotoxicological studies with Icelandic scallop are relatively scarce and few report on biomarkers. However, some authors have used *C. islandica* to study biological responses in conditions where this species lives (Camus et al., 2002; Regoli et al., 2000); due to their relatively wide distribution in Arctic waters, Icelandic scallops represents a promising substitute sentinel species for the northern marine areas where mussels are not present.

One mechanism of toxicity after exposure to PAH appears to relate to the production of reactive oxygen species (ROS) as by-products of metabolism (Digiulio et al., 1989; Livingstone, 2003). These highly potent molecules can react with important macromolecules like DNA, proteins and lipids. The consequences of ROS for cell components and their functioning can be at times critical for individuals. Aerobic cells have developed a variety of defences to protect themselves against these oxyradical products. For example, several enzymes exist that can counteract the pro-oxidant challenge, but overwhelming is also possible (Regoli et al., 2004). To evaluate the mussels' antioxidant status, common biomarkers include the level of glutathione, gluathione-*S*-transferase, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Here, we selected two of these biomarkers to estimate changes in the redox status: gluthathione-*S*-transferase (GST) and catalase (CAT). Also, the total oxidative scavenging capacity (TOSC) was used as an overall redox status parameter.

Catalase is considered a true oxidative scavenger enzyme which removes the potential damage of hydrogen peroxide by catalysing the reaction $2H_2O_2 \rightarrow 2H_2O + O_2$ (Digiulio et al., 1989; Orbea et al., 2002) while GST is more involved in the phase II detoxification process by adding gluthathione to reactive metabolite compounds thereby facilitating their excretion. GST contributes significantly in the defence against oxidative damage of DNA and lipids, and is used as a biomarker of organic contamination in bivalves (Bocquene et al., 2004; Devier et al., 2005; Moreira and Guilhermino, 2005). Compared to individual antioxidant enzymes, the TOSC assay can readily be related to different forms of ROS and has a better predictive value to evaluate the overall redox status (Regoli et al., 1998, 2002). The changes in TOSC have been associated with damages at the cell level such as DNA damage and lysosome membrane stability in fish and in mussels (Regoli et al., 2003, 2004).

At the cellular level, the lysosomal membrane stability assayed by the neutral red retention time (NRRT) is easy to perform on haemocytes of mussels. It is used as a general low-cost marker to detect impairments of the functional integrity of cells (Livingstone et al., 2000). Lysosomes are cellular organelles and in mussels, they play an important role in several biological pathways, including the immunological defence system (Moore and Willows, 1998). Furthermore, there appears to be a good link between the impairment of lysosomal membrane, oxidative stress, genotoxicity and fitness parameters (Moore et al., 2004). The comet assay is a relatively simple and sensitive technique for determining DNA strand breaks in individual cells. The assay is used as a marker of genetic impairment and has been used to assess DNA damage in various marine organisms exposed to various types of environmental toxicants (Lee and Steinert, 2003; Mamaca et al., 2005; Mitchelmore and Chipman, 1998; Perez-Cadahia et al., 2004).

In this paper, the individual responses of each of the abovementioned biomarkers were first investigated in the two mollusc species and related to several exposure concentrations of crude dispersed oil. Statistically significant responses compared to control individuals were analysed. Secondly, multivariate analysis was applied as a way to integrate all biomarker responses into a overall "health" assessment index and to examine the combined biomarker patterns for the two species.

The aims of this study were: (i) to examine the biomarker responses obtained in *M. edulis* and *C. islandica* using appropriate laboratory conditions for the two species and (ii) to conclude on the suitability of the selected biomarkers for environmental monitoring in marine regions where oil and gas activities exist or are prospected. The two species were exposed to dispersed crude oil originating from the North Sea (*M. edulis*) or the Barents Sea (*C. islandica*).

2. Materials and methods

2.1. Origin of the organisms

Blue mussels *Mytilus edulis* were collected in December 2002 at a farm located close to the Lysefjord (Rogaland county, Norway) and purchased at Aspøy Skjell og Produktutvikling AS (Hundvåg, Norway). At the research centre facility (Akvamiljø AS, Randaberg, Norway), they were transferred to a 350 l tank with running 34‰ seawater at a temperature of 7 ± 0.5 °C. Mussels were used in the experimental system after ca. 2 weeks. No mortality was recorded.

The Icelandic scallops *C. islandica* were collected by a bottom dragger during a cruise with R/V Jan Mayen around Spitzbergen carried out in September 2003. After sampling, they were kept on board the ship with running sub-surface seawater. Thereafter, they were shipped by plane to our facility and immediately transferred to a tank filled with 200 l running seawater at 4 ± 0.5 °C. *C. islandica* were kept ≈4 weeks before they were used in the experiment. During this quarantine period the mortality was 13% of which the largest proportion died during the week following arrival.

The seawater was pumped directly from 75 m depth in the Byfjord close to our facility and sand-filtered before use in the experimental system. Both molluscs were fed regularly a mixture of live *Isochrysis galbana*, *Rhodomonas baltica* and *Skeletonema costatum* during the maintenance period.