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Investigation on the proteome response of transplanted blue mussel (*Mytilus* sp.) during a long term exposure experiment at differently impacted field stations in the German Bight (North Sea)

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ABSTRACT

In a pilot field study the proteome response of *Mytilus* sp. was analyzed in relation to the concentration of different trace metal contaminants. Over a period of eight month test organisms have been exposed at a near-shore station in the anthropogenic impacted estuary of the river Elbe and at an off-shore station in the vicinity of the Island of Helgoland in the German Bight (North Sea). The stations differ in their hydrological as well as chemical characteristics. The physiological biomarkers, such as condition index which have been continuously monitored during the experiment clearly indicate the effects of the different environmental conditions. Multiple protein abundance changes were detected utilizing the techniques of two dimensional gel electrophoresis (2dGE) and consequently proteins arising as potential candidates for ecotoxicological monitoring have been identified by MALDI-ToF and ToF/ToF mass spectrometry. Different cytoskeletal proteins, enzymes of energy metabolism, stress proteins and one protein relevant for metal detoxification have been pointed out.

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1. Introduction

Mussels are well established indicators for environmental pollution in marine and coastal habitats due to their sessile and filter feeding existence and their ability to bioaccumulate a multitude of either trace metal as well as organic contaminants. In consequence, their body burdens provide integrated information on the pollution status of the near surrounding environment. Especially the utilization of transplanted mussels compensates the biological diversity and scarcity related with the use of natural mussel populations as indicators, which often complicates the final data interpretation as well as the wide spread application of such approaches. The purposeful deployment of transplanted mussels in an in situ experiment provides important information on the bioavailability of contaminants at one hand and associated possible toxic effects on the other hand and it combines the advantages of realistic environmental and semi-controlled experimental conditions (Salazar and Salazar, 1995). The usefulness of applying caged mussels for biomonitoring purposes have been shown in several studies (Bodin et al., 2004). Along the French Mediterranean coast differently polluted areas which were not sampled before could be distinguished based on such approach. A good agreement with the contamination level of parallelly investigated wild population was demonstrated (Andral et al., 2004). A further successful example for a caged mussel experiment to distinguish polluted from less-polluted sites at the Greek Mediterranean coastline is described by Tsangaris et al. (2010). Bocchetti et al. (2008) applied caged mussels for an integrated biomonitoring study on the impact of dredging and disposal operation in harbor areas, and they showed toxic effects related to elevated levels of inorganic and organic contamination in the tissues of the caged mussels.

A long-term biomonitoring study with transplanted *Perna perna*, integrating data on bioaccumulation of different classes of pollutants with data on biomarker related to defense mechanisms,

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pointed out differences between sampling stations, seasons and critical areas in terms of contamination levels (Pereira et al., 2012).

Even short-term exposure experiments with transplanted mussels as bioindicators were performed to assess the water quality by separating impacted areas based on physicochemical and biochemical parameters (Giarratano et al., 2010).

In the North Sea area the pilot study performed in the BECPELAG project is an excellent example for the methodological performance as well as the integrative character of such caged mussel approach (Hylland et al., 2006).

Classically selected physiological and biochemical parameters at the organismal and cellular level are utilized as responsive elements (biomarker) to indicate chemical stress. Due to the multitude of potential contaminants in the marine and coastal environment and due to the complexity of organism responses the analysis of a selected set of biomarkers at different levels of biological organization have been strongly recommended rather than focusing on a single marker of effects. Several suggestions for a rational integrated assessment of biomarker responses have been made and applied for environmental monitoring projects in order to achieve a comprehensive risk assessment and in consequence to finally allow a description of the environmental health status (Beliaeff and Burgeot, 2002; Brooks et al., 2009; Gagne et al., 2008; Narbonne et al., 2001; Yeats et al., 2008).

Although biochemical and cellular events tend to be more sensitive than stress indices at the whole organism level, it is often difficult to find correlations with ecological impacts (Amiard-Triquet and Pavillon, 2004; Viarengo et al., 2007). However, it may provide an early warning of higher order biological effects.

Since some years state-of-the-art proteomic techniques have been providing the opportunity to observe a suite of responses in form of protein expression signatures (PES) at the molecular level. The pattern of molecular biomarkers plays an important role in understanding the relationships between exposure to pollutants and possible responses, in revealing modes of effects and in identifying key pathways in the development of diseases. Major tools of proteomics are two dimensional gel-electrophoresis (2dGE) or other high resolution multidimensional protein separation techniques which provide a global expression pattern of the proteins present in a sample. These techniques are combined with different mass spectrometric techniques which allow the identification of the individual regulated proteins.

Environmental proteomics examines how multiple abundance changes are associated with a contamination which is suspected to have a detrimental effect (Sanchez et al., 2011). Although the utilization of such techniques represents a promising approach for a comprehensive assessment of water quality, there are only few fundamental proteome analysis studies with mussels as bioindicators available in literature and most of them are based on laboratory exposure experiments (Apraiz et al., 2006; Campos et al., 2012; Dondero et al., 2010; Jonsson et al., 2006; Liu et al., 2012; Lopez et al., 2002; Rodríguez-Ortega et al., 2003; Shepard et al., 2000).

The obtained data have to be carefully analyzed according to methods consistency, reproducibility, statistical significance and accuracy to balance the biological variance, to filter out pronounced effects and to process qualified biomarker. Facing the challenge related with the transfer from laboratory to field samples, marine proteomics is an expanding and promising molecular research tool (Slattery et al., 2012).

Within this background the present study describes the results of a long-term field exposure experiment with transplanted *Mytilus* sp. at differently impacted coastal areas of the German Bight in the North Sea using a combined approach which uses either chemical and biochemical analysis of inorganic contamination as well as the analysis of a molecular response in mussel tissue. The main objective was to demonstrate the suitability of using PES to distinguish different anthropogenic impacted areas and to identify major differently expressed proteins as potential biomarker.

2. Material and methods

2.1. Field exposure and sampling

Cohorts of mussels of the same origin (obtained from commercial fisheries at the Island of Sylt, Germany) were deployed in cages at two different field stations; one located at the Island of Helgoland, German Bight and the other at the estuary of the river Elbe in Cuxhaven, Germany from May 2011 to January 2012. Oceanographic data such as Sea Surface Temperature (SST) and Salinity were continuously recorded using the Coastal Observing System for Northern and Arctic Seas COSYNA powered by the Helmholtz-Zentrum Geesthacht Centre of Materials and Coastal Research. At the station in Cuxhaven the salinity ranges vary between 10 and 25. The mussels are continuously submersed. The salinity at the off-shore station Helgoland was constantly above 30. The SST was slightly higher at the station Cuxhaven at the beginning of the field exposure experiment with 17 °C compared to 14°C at Helgoland. A maximum of 20 °C at Cuxhaven and 17 °C at Helgoland was reached in August and the temperature dropped to 5 °C in Cuxhaven and 7 °C at Helgoland in December. Mussel sampling occurred every 6 weeks over an exposure period of eight month. The sampling at Helgoland was done by the group of Scientific Diving, Alfred Wegener Institute for Polar- and Marine Research Bremerhaven, Station Helgoland. After the recovery of the mussels from the submerse station, the mussels were placed in filtered Helgoland seawater in a flow through tank over night and shipped under cooled conditions to Cuxhaven. The cohort of mussels at Cuxhaven was accessible via an elevator construction and both sample groups were transported to the laboratory simultaneously wetted and cooled with respect to minimize stress related effects. Due to logistical circumstances the mussels from Helgoland were transported about 4 h longer than the mussels from Cuxhaven. The organisms of both groups were kept wetted and cooled until preparation.

2.2. Physiological parameter

Composite samples each composed of ten organisms were used for the measurement of the physiological parameters Condition Index (CI tissue dry weight/shell dry weight) and Gonadosomatic Index (GSI wet weight gonads/wet weight soft tissue x 100) (Pampanin et al., 2005). After the sample preparation the tissue and shells were dried by lyophilization for five days. The protein contents of gill extracts were measured after purification using Micro-Bio-Spin 6 columns (Biorad, Munich, Germany) by Bradford Protein Assay and bicinchoninic acid (BCA) assay (Thermo Scientific Pierce ™, Dreieich, Germany) using Bovine serum albumin (BSA ACS chemicals) as protein standards (Bradford, 1976). Protein values were calculated as mean of one Bradford and two BCA assay with 3 replicates each.

2.3. Trace element analysis of the whole mussel tissue

To avoid any contamination of the tissue samples every mussel was flushed with MilliQ water before the opening of the shell. A cleaned ceramic knife was applied for the opening as well as for the tissue removal from the shells in order to minimize trace element contamination. After the opening of the shell the inside of the shell as well as the whole soft tissue were flushed with MilliQ water to

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