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Detoxification and protein quality control markers in the mussel *Mytilus edulis* (Linnaeus) exposed to crude oil: Salinity-induced modulation

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ABSTRACT

Marine and coastal ecosystems are influenced by oil from chronic contamination or sporadic oil spills. An oil spill was simulated in an aquarium-based experiment designed to reproduce interactions of crude oil with inert environmental components, particularly adhesion on shore gravel and dissolution in sea water. Total experimental oil concentrations were in the range of comparable hydrocarbon concentrations following an oil spill. Furthermore, the possible interaction of a chemical (anthropogenic) stressor, such as oil PAHs, and a "natural" stressor like desalination, was simulated. In order to assess the biological effects of crude oil contamination and desalination (each individually and in combination) on the blue mussel *Mytilus edulis* L., biochemical responses were estimated including: detoxification capacity by glutathione-S-transferase (GST) activity, reduced glutathione (GSH) level, and protein quality control by autophagy-related proteases cathepsin B (CatB), cathepsin D (CatD), and calcium-dependent calpain-like proteases. Oil treatment stimulated defense system response in the mussels with primary effects on GST and protease-mediated reactions such as the activation of CatB, CatD, and calpains. Most of biomarkers responded to oil in a dose- and time-dependent manner. Additional environmental stress, such as desalination, promoted the oil-induced activation of GST and CatD while resulting in a delay or impairement of the defense response to oil by GSH and proteases CatB and calpains. Thus, biomarker data shows that combined effects of oil compounds and desalination can be realized in both a synergistic and an antagonistic manner. The evaluated interaction between oil pollution effects and sub-optimal salinity on *M. edulis* indicates the potential risk of maladaptation to the biota of estuaries.

1. Introduction

Increased oil-related activities has resulted in marine and coastal ecosystems being subjected to substantial contamination from chronic conditions or sporadic spills (Dahle et al., 2009; Mitsch, 2010; Neuparth et al., 2012; Mitchell et al., 2015). The blue mussel, *Mytilus edulis* L. (Mollusca, Bivalvia), is a sessile and filter feeding organism, known as a sentinel of marine pollution, including oil contamination (Burns and Smith, 1981; Apraiz et al., 2006). Mussels readily accumulate oil compounds (Valavanidis et al., 2008; Arias et al., 2009); bioaccumulation of associated polycyclic aromatic hydrocarbons (PAHs) is determined by the availability of dissolved PAHs in surrounding water, which in turn is dependent on ambient salinity (Neff and Anderson, 1981). Mussel biochemical responses have been successfully used as biomarkers for assessing the potential toxic effects of PAHs in marine coastal areas (Cajaraville et al., 2000; Sureda et al., 2011). The glutathione/glutathione-S-transferase (GSH/GST) system and lysosomal-autophagic system are involved in response systems associated with PAHs and have been applied as biomarkers (Cajaraville et al., 1995; Regoli et al., 2004; Moore et al., 2007; Sureda et al., 2011; Higgins and Hayes, 2011; Luchmann et al., 2014; Lysenko et al.,

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2014). Mussels must cope with severe background environmental stressors such as fluctuations in temperature, salinity, nutrients, and hypoxia (Wolfe, 1986; Hochachka and Somero, 2002; Bussell et al., 2008) and must continually compensate suboptimal conditions by utilizing diverse physiological abilities. Exposures to a pollutant may interfere with these compensatory responses; therefore, it is presumed that the combined effects of naturally-encountered stressors and a toxicant will exceed those that expected from either of them alone (Hamer et al., 2008; Holmstrup et al., 2010; Whitehead, 2013). Based on evaluation by Holmstrup et al. (2010), the effects of natural stressors combined with a variety of toxicants are realized in both a synergistic (more than 50% of cases) and an antagonistic manner. Environmental stressors affect the biological parameters used for pollution assessment (Cajaraville et al., 1995, 2000; Sarkar et al., 2006; Hamer et al., 2008; Sokolova et al., 2012); however, little is known about biomarker variability associated with salinity fluctuations. Therefore, sub-optimal salinity directly influences the oil uptake in *M. edulis* while indirectly affecting oil-induced biomarker response (Ramachandran et al., 2006; Zanette et al., 2011).

To simulate pollutant exposure under a realistic environmental context, the effect of oil contamination on mussels was studied under normal and sub-optimal salinity in an aquarium-based experiment. These parameters are representative of the coastal estuaries, including those in the Kandalaksha Gulf in the White Sea (Savinov et al., 2000; Fokina et al., 2014; Lysenko et al., 2014). This study aims to demonstrate and distinguish the oil- and desalination-induced responses in mussels and evaluate potential stressor interactions. To assess independent and combined biological effects of crude oil and desalination, specific biomarkers indicative of both organic contamination and oxidative stress were assessed in *M. edulis* gills and hepatopancreas. Biomarkers investigated included components of antioxidant defense and detoxification systems, including glutathione-S-transferase (GST; EC 2.5.1.18) activity, reduced glutathione (GSH) content, in addition to protein quality control enzymes such as autophagy-related proteases, cathepsin B (CatB; cysteine-type, EC3.4.22.1) and cathepsin D (CatD; aspartic-type, EC 3.4.23.5), and calcium-dependent calpain-like proteases (referred as calpains; EC 3.4.22.17).

2. Materials and methods

2.1. Sampling of mussels

Blue mussels were collected in September and October of 2013 from mussel culture facilities at 2.0 m depth (66° 33′ N, 33° 64′ E; Kruglaya Bight, Chupa Bay, Kandalaksha Gulf of the White Sea, N–W Russia). Temperature and salinity at the sampling site were measured *in situ*, simultaneous to mussel collection: 8.0 °C and 24.2 psu, respectively. The sampling point used was isolated from the primary sources of oil contamination in the Kandalaksha Gulf and seawater in the area was regarded as relatively unpolluted. Mussels were sorted according to size and age; mussels of shell length 60.2 ± 2.9 mm and between 6 and 7 years of age were maintained in plexiglass aquaria (25 individuals per 20 L aquarium) with aerated sea water. The experimental period was during the reproductive resting of the mussels (stage 0, no gametes in gonads) and sex determination was therefore not possible. Before initiating experimentation, mussels were randomly assigned to two sample groups subjected to either normal (25 psu) or desalinated (15 psu) sea water for 10 days at 10 °C with a 12:12 (light:dark, h) regime (Osram L36W/640, 2850 lm, Russia). Water in the aquaria was partially (10 L) replaced daily.

2.2. Experimental design

Experimental procedures were carried out at the “Kartesh” Skarlato White Sea Biological Station (Zoological Institute of Russian Academy of Sciences). Experimental oil spills were simulated in the aquaria through initial adhesion of crude oil on shore gravels and subsequent dissolution of unbound oil fractions in replaceable seawater. In order to obtain oil emulsion and avoid layering, 100 mL of Surgut oil were dissolved in 900 mL sea water and mixed thoroughly by shaking for 10 min. The resultant mixture was added to each gravel-filled tank (5 kg of uniform-sized gravels per tank) so that oil amounts averaged 1, 5, or 50 mL per tank, corresponding to low (L), medium (M), and high (H) oil treatments, respectively. Tanks were then filled with 25 psu or 15 psu sea water for final crude oil concentrations of 0.05, 0.25, and 2.50 mL/L), and all with normal and reduced salinity. After 24 h, 10 L of oil-containing water from each tank was added to each aquarium containing sample mussels; the removed volume was substituted by clean sea water to simulate a contact of tidal flow with oil-contaminated seashore gravel. These procedures were repeated daily throughout the experiment. Water samples for PAH quantification were taken from tanks filled with oil-contaminated gravel (Initial point) and from each aquaria at exposure times of 1, 3, and 10 days.

2.3. Chemical and biomarker analyses

Salts, chemical reagents, protease inhibitors, protein substrates, molecular weight markers, and dyes were purchased from Sigma–Aldrich (St Louis, MO, USA) and of analytical grade. Crude oil was obtained from Surgut oil producer. Technical facilities of the Equipment Sharing Centre of the Institute of Biology, KarRC of RAS were used.

2.3.1. Water analyses

Water analyses for PAH were performed in the Institute of Northern Water Problems, KarRC RAS. Oil components were extracted from water samples by tetrachloromethane (CCl_4) and quantified by infrared (IR) spectrometry using Fourier transform infrared spectrophotometer IRPrestige-21 (Shimadzu, Japan). Results were expressed as total PAH content (mg/L).

2.3.2. Tissue sampling

Tissue sampling was performed one day prior to the addition of oil-contaminated water (control group) and at 1, 3, and 10 days following exposure. The hepatopancreas and gill samples intended for biochemical analysis were rapidly excised and flash-frozen in liquid nitrogen; samples were then transported to the laboratory and maintained at −80 °C (UF 240-86 E freezing chamber; Snijders Scientific, The Netherlands), and analyzed within 2 months.

2.3.3. Glutathione S-transferase activity assay

Mussel tissues (0.1 g) were individually homogenized in a 0.125 M potassium phosphate buffer. Homogenates were centrifuged at 100,000 g for 60 min to remove cell debris. Resulting supernatants were used for enzyme activity assays. GST activity was determined according to a procedure described by Habig et al. (1974); the 1 mL reaction mixture was as follows: 0.125 M potassium phosphate buffer (pH 6.5), 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), 1 mM reduced glutathione (GSH) and 100 μL of supernatant. Enzymatic activity was determined by kinetic measurement of absorbance at 340 nm using a SP-2000 spectrophotometer (OKB Spectrum, Russia) at 20 °C. Activity was expressed as nM GSH-CDNB produced per min and per mg protein (nM/min/mg).

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