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Assessment of the fitness of the mussel *Mytilus galloprovincialis* two years after the *Hebei Spirit* oil spill

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ABSTRACT

In December 2007, >150 km of the West coast of Korea were heavily polluted by crude oil leaked from the oil tanker *Hebei Spirit*, leading to mass mortality of bivalve mollusks on the intertidal areas. Two years after, mussels *Mytilus galloprovincialis* were collected from two impacted sites to investigate sub-lethal effects of the oil spill. Tissue content in polycyclic aromatic hydrocarbons (PAHs), hemocyte parameters, reproductive status and energetic reserves were analyzed. PAHs in tissues of mussels as well as hemocyte parameters were not different between impacted and control sites. Energetic reserves were altered in mussels from the impacted sites. Glycogen content remained low at polluted sites, whatever the season. Two years after the *Hebei Spirit* oil spill, mussels then presented altered energetic metabolism. Further investigations are thus warranted to monitor the sustainability of mussel populations on the oil spilled West coast of Korea.

1. Introduction

In December 2007, approximately 10,900 tons of crude oil leaked from the oil tanker *Hebei Spirit* (Kim et al., 2010), covering >150 km of the West coast of Korea (UNEP/OCHA, 2008). Taean shoreline, the most heavily damaged area (Fig. 1), hosts numerous aquaculture farms and is home for various intertidal bivalve mollusk species such as the Manila clam *Ruditapes philippinarum*, the Pacific oyster *Crassostrea gigas* and the Mediterranean mussel *Mytilus galloprovincialis*. On the intertidal areas heavily covered with crude oil, mass mortality of bivalves occurred (MLTMA, 2009). Due to the oil pollution, aquaculture and fishery activities in Taean area were therefore officially suspended (MLTMA, 2009).

Two years after the accident, elevated concentrations of residual crudes and polycyclic aromatic hydrocarbons (PAHs) were still present in the sediment, particularly in subsurface layers (MLTMA, 2009, 2010; Hong et al., 2012). Burrowing animals such as Manila clams, that remained permanently exposed for two years, displayed altered internal cellular defense and energetic metabolism (Hong et al., 2016). In water column however, levels of PAHs rapidly decreased to become undetectable nine months after the oil spill (MLTMA, 2009, 2010; Hong et al., 2012). The initial acute crude oil pollution as well as the chronic presence of PAHs in the sediments may have durably altered local environmental biotic and abiotic variables (NRC, 2003; Chang et al., 2014; Ozhan et al., 2014). Although sessile benthic animals such as oysters and mussels were no longer directly exposed to oil contaminants, potential sub-lethal effects still needed to be determined.

In marine bivalves, hemocytes are cells freely circulating in hemolymph and infiltrating in tissues. Among several physiological functions, hemocytes are responsible for internal defense, from immunity to detoxification of xenobiotics (Cheng, 1981; Chu, 2000; Donaghy et al., 2009a). Hemocytes are sensitive to external and internal disturbances and have often been used as proxy for the health status of bivalves (Auffret, 2005; Donaghy et al., 2009a; Girón-Pérez, 2010; Ellis et al., 2011). In situ and in vitro exposure of marine bivalves to crude oil and PAHs can result in sub-lethal effects on the internal defense system (Dyrynda et al., 2000; Donaghy et al., 2010; Hong et al., 2016). However, hemocyte parameters are not always impacted by oil exposure. For instance, hemocytes of *M. galloprovincialis* were not affected by a 4-month laboratory exposure to oil from the Prestige oil spill (Ordás et al., 2007). Also, one year after the Erika oil spill, depressed immune system was only detected in Pacific oysters from one of the numerous damaged sites (Auffret et al., 2004). Potential sub-lethal effects of oil spills on marine bivalves are therefore challenging to predict and require further investigation.

Prevalence and intensity of parasitic infections in bivalves are known to increase with pollutant exposure, including oil and PAHs (Barszcz et al., 1978; Kim and Powell, 2007; Kim et al., 2008). Furthermore, homeostasis, metabolism and physiology of animals can be altered by chronic exposure to environmental pollution. Particularly, energetic reserves and reproduction capacity, which are primordial for

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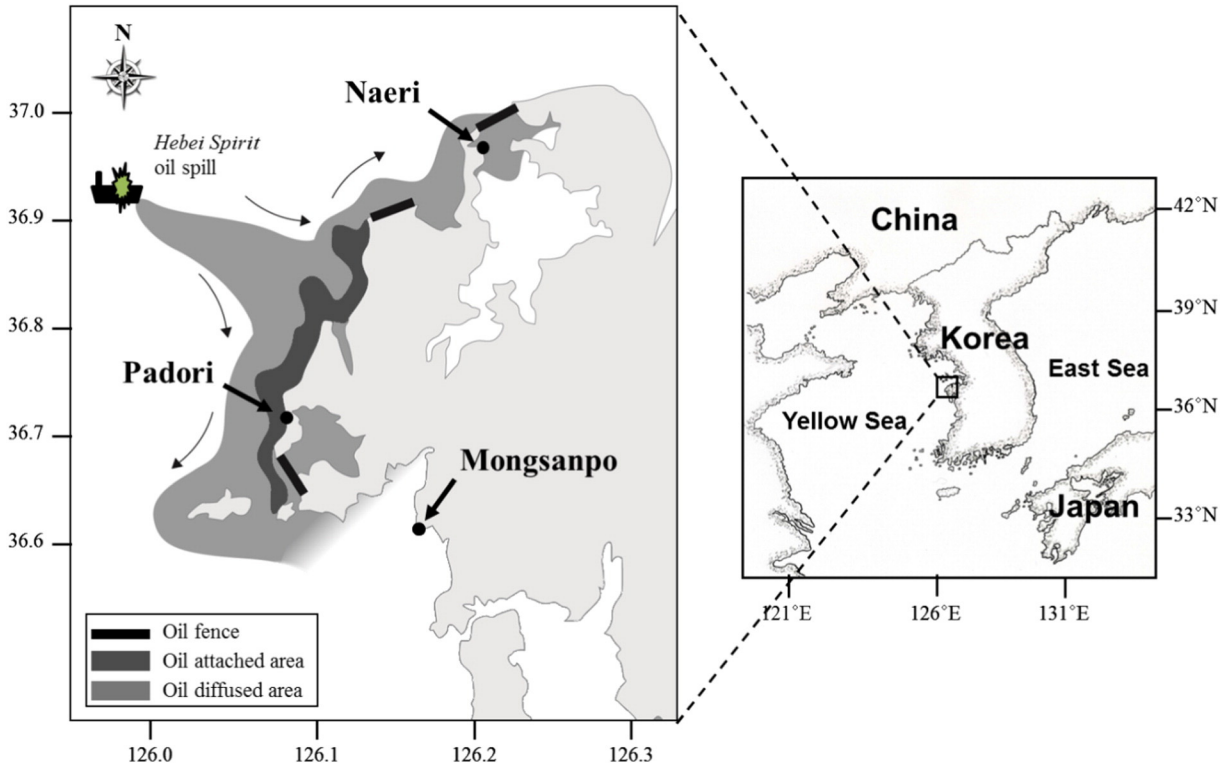

Fig. 1. Sampling sites of *Mytilus galloprovincialis*. Mussels were collected from two oil spill impacted sites, Naeri and Padori, and one control site, Mongsanpo.

sustainability of local populations, may be influenced by chemical pollutant exposure and alteration of environmental variables (Aarab et al., 2004; Voets et al., 2006; Tlili et al., 2011).

The objective of the present study was to determine the overall fitness of the mussel *M. galloprovincialis*, two years after the *Hebei Spirit* oil spill, through a comprehensive approach. In summer (June 2009) and winter (January 2010), mussels from two impacted sites on Taean coast were examined for tissue content in PAHs, hemocyte parameters, parasitic infections, reproduction status and energetic reserves.

2. Material and methods

2.1. Study area

Adult mussels *M. galloprovincialis* were collected from the oil spill impacted sites of Naeri and Padori on Taean coast (Fig. 1). As control, mussels were collected from Mongsanpo (Fig. 1), an area undamaged by the oil spill (MLTMA, 2009, 2010). The levels of 16 PAHs and alkylated PAHs in water and sediments at the different sites have been monitored by the Oil and POPs Research Group of Korea Institute of Ocean Science and Technology (KIOST). PAH levels in the sediment and water of both spilled sites were high during the early stage of the oil spill. PAH levels in water column rapidly decreased while it took one year after the accident to reach the lowest plateau in the sediment. Control site (Mongsanpo) was not impacted by the oil spill: PAH levels remained very low from the early stages of the accident to sampling times (MLTMA, 2009, 2010).

2.2. Sampling effort

In June 2009 and January 2010, *M. galloprovincialis* were collected from the spilled sites Naeri and Padori, and from the control site Mongsanpo (Fig. 1). Arrival upon laboratory, mussels were placed in 100 L seawater tanks (15 °C and 32 psu) for 24 h to minimize the potential disturbance from transportation. For the analyses, a minimum of 30 mussels were randomly selected from each site.

2.3. Hemocyte parameters

2.3.1. Hemolymph collection

Hemolymph was withdrawn from the adductor muscle, microscopically checked for purity, filtered (80 μm nylon mesh) and held on ice until flow cytometry analysis. Hemocyte parameters were analyzed using a FACS Calibur flow cytometer (Becton-Dickinson, USA). Protocols were adapted from Donaghy et al. (2009b). All subsequent analyses were conducted individually.

2.3.2. Hemocyte mortality

Hemocyte mortality was determined using propidium iodide (PI; Sigma-Aldrich, USA) staining procedure. Hemolymph was mixed with an equal volume of anti-aggregant solution (AASH, 2.5% NaCl, 1.5% EDTA in 0.1 M phosphate buffer, pH 7.4) containing PI (final concentration 20 μg/mL) and incubated for 10 min in the dark at room temperature. Hemocyte mortality was determined as the percentage of PI-positive cells.

2.3.3. Percentage of granulocytes

The percentage of granulocytes was determined using SYBR Green I (Sigma-Aldrich, USA). Hemocytes were fixed in 3% formalin solution containing SYBR Green I and incubated for 90 min in the dark at room temperature. Granulocytes were discriminated based on higher side scatter (SSC) compared to hyalinocytes. The percentage of granulocytes was determined among the total quantity of hemocytes.

2.3.4. Phagocytic activity

Phagocytosis activity was investigated through the capacity of hemocytes to internalize fluorescent latex beads (2.0 μm in diameter, Polysciences Inc., USA). Hemolymph was mixed with an equal volume of 2% fluorescent beads diluted in sterile seawater and let for 2 h in the dark at room temperature. Phagocytosis activity was calculated as the percentage of hemocytes that engulfed three or more beads.

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