Available online at www.sciencedirect.com
ScienceDirect
Environment International 34 (2008) 483–489
ENVIRONMENT INTERNATIONAL
www.elsevier.com/locate/envint

Immediate biomarker responses to benzo[a]pyrene in polluted and unpolluted populations of the blue mussel (*Mytilus edulis* L.) at high-latitudes

Halldór P. Halldórsson [a,b,*], Maurizio De Pirro [c], Chiara Romano [d], Jörundur Svavarsson [a,b], Gianluca Sarà [e]

[a] *Institute of Biology, University of Iceland, Askja—Natural Science Building, Sturlugata 7, 101 Reykjavík, Iceland*
[b] *Suðurnes University Research Centre, University of Iceland, Garðvegur 1, 245 Sandgerði, Iceland*
[c] *Accademia Mare Ambiente, Lungomare dei navigatori 44, 58019 Monte Argentario, Italy*
[d] *IAMC-CNR-IRMA, Via da Terrazzano, 17, 91011 Castellammare del Golfo, Italy*
[e] *Dipartimento di Biologia Animale, Università di Palermo, Via Archirafi, 18, 90123 Palermo, Italy*

Received 5 April 2007; accepted 6 November 2007
Available online 11 December 2007

Abstract

Immediate biomarker responses of two high-latitude populations of the blue mussel *Mytilus edulis* to benzo[a]pyrene (B[a]P) were evaluated. Mussels collected from a clean and a polluted site in southwest Iceland were exposed to the nominal dose of 100 μg B[a]P L^{-1} for 3 h, after 4 days of acclimatization in clean seawater. To test the sensitivity to the toxicant and immediate biological responses, the following biomarkers were used: DNA single strand breaks, heart rate and feeding rate.

All the biomarkers revealed differences between the study sites. Irrespective of the origin of the organisms, the short time exposure to the high B[a]P concentration did not induce DNA single strand breaks or significantly affect the feeding rate. However, the heart rate results showed significantly different responses. The mussels from the polluted site (Reykjavík harbour) increased their heart rate when exposed to B[a]P, while no difference was observed between the heart rate values of the individuals from the clean site (Hvassahraun). The mussels seem to sense the pollutant they have been previously exposed to, and their acute response indicates physiological adaptation to the polluted environment. The results indicate limited sensitivity and temporal predictivity, i.e. transient measurable changes of these biomarkers, as well as showing that the background of the organisms should be considered when evaluating short-term biomarker responses to contaminants.

Keywords: *Mytilus edulis*; Benzo[a]pyrene; Biomarkers; Sensitivity; Heart rate; Feeding rate; Genotoxicity

1. Introduction

The effects of oil compounds, in particular polycyclic aromatic hydrocarbons (PAHs), on marine organisms have recently received much attention due to several large oil spills resulting in extensive environmental and economic effects (AMAP, 2002; Albaigés et al., 2006; Loureiro et al., 2006). The continued threat of oil spills may in the future reach new dimensions. Recent climate change has resulted in decrease of the ice sheet of the Arctic Ocean (ACIA, 2004, 2005), and it can be foreseen that within several years large oil tankers can use the North-east passage between Asia and Europe, i.e. crossing the Arctic Ocean north of Russia (UNEP, 2004). This, in addition to increasing gas and oil exploration in the Arctic, increases the need to further understand the effects of oil compounds and PAHs on organisms at high-latitudes.

Effects of PAHs on organisms are extensive on various organism levels, including genotoxic, biochemical and physiological effects (Livingstone et al., 1985; Lowe et al., 2006). The responses or tolerance of organisms to PAHs can however vary considerably, even for the same species. Partly, this can be due

* Corresponding author. Institute of Biology, University of Iceland, Askja—Natural Science Building, Sturlugata 7, 101 Reykjavík, Iceland. Tel.: +354 5255226; fax: +354 5254069.
E-mail address: halldor@hi.is (H.P. Halldórsson).

0160-4120/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2007.11.002

to the fact that PAH mixtures can vary significantly within the geographical range of a species (Anderson and Lee, 2006; Skarphéðinsdóttir et al., 2007). Populations have also been shown to respond differently to PAHs, possibly reflecting the pre-exposure history and/or heritable, genetic changes in populations chronically exposed to mutagenic xenobiotics (Courtenay et al., 1999; Ma et al., 2000). Additionally, PAH levels and the subsequent effects on organisms, such as DNA adducts, may vary considerably between seasons at high-latitudes (Skarphéðinsdóttir et al., 2005). Little is still known of how responses, sensitivity and tolerance to PAHs are influenced by exposure to the contaminants. The estimation of PAH exposure and effects in invertebrate species remains problematic despite intensive research effort (Galloway, 2006).

The aims of this study were to evaluate immediate responses and sensitivity to benzo[a]pyrene of blue mussels (*Mytilus edulis* L.) coming from two different high-latitude environmental conditions: a polluted site (Reykjavík harbour) and a clean site (coastal site of Hvassahraun). Furthermore, to evaluate the heart rate as a potential monitoring tool at high-latitudes. The PAH compound benzo[a]pyrene (B[a]P) was selected as the test pollutant because it is one of the most harmful polycyclic aromatic hydrocarbons (IARC, 1987), and has also been reported in high concentrations in Reykjavík harbour (Ericson et al., 2002; Halldórsson et al., 2005). It is a potent genotoxic chemical, readily accumulated in the tissues of bivalve molluscs. Various effects of B[a]P on the blue mussel have been recorded, both at cellular and individual level (e.g. Eertman et al., 1995; Garcia and Livingstone, 1995; Large et al., 2002; Skarphéðinsdóttir et al., 2003; Moore et al., 2006).

To test the sensitivity and immediate metabolic responses of the blue mussel to B[a]P we used a suite of biomarkers, as widely recommended to get a holistic view on the subject (Hebel et al., 1997; Nicholson and Lam, 2005) and proposed for rapid assessment of marine pollution and coastal marine ecosystem health (Bowen and Depledge, 2006). We used the following biomarkers: heart rate, feeding rate (i.e. clearance rate; part of the biomarker scope for growth) and DNA single strand breaks, measured by means of the comet assay. These biomarkers have been widely applied to molluscs for ecotoxicological and monitoring purposes, both in laboratory and field studies (e.g. Widdows et al., 1997; Bolognesi et al., 2004; De Pirro and Marshall, 2005; Emmanouil et al., in press). They are non-specific biomarkers responding to a wide range of contaminants, which selection was based partly on the background information on the use of these biomarkers at high-latitudes (feeding rate, DNA strand breaks; Halldórsson et al., 2004, 2005). The high nominal B[a]P concentration of 100 μg L^{-1} was chosen to stimulate biological responses to be evaluated in the time-span of 3 h.

To our knowledge, short or long term effects of B[a]P on the heart rate of mussels have not earlier been evaluated, but cardiac activity in bivalve molluscs has recently been suggested as a good biomarker of the metabolic status, reflecting general environmental stress (Galloway et al., 2004; Bowen and Depledge, 2006).

2. Materials and methods

2.1. Study area and sample collection

The blue mussels (*M. edulis* L.) were collected at two sites in Southwest Iceland, i.e. at a clean site and at a polluted one. The clean site is at Hvassahraun (64°01′11′′N, 22°09′31′′W) on the northern side of the Reykjanes peninsula. This site has been shown to be pristine and organisms from this site have frequently been used in monitoring programmes and as reference material in experiments (Yngvadóttir et al., 2006; Da Ros et al., 2007). No ship traffic or industrial activity is close to this site (<10 km).

The contaminated site is the inner side of Reykjavík harbour (64°09′01′′N, 21°56′05′′W). This harbour has extensive shipping activity and previous studies have shown high levels of PAHs in the tissues of the blue mussel. At the same location resident mussels had for example ∑16 PAHs of 27 μg g^{-1} lipid, and transplanted mussels had ∑32 PAH levels as high as 56 μg g^{-1} lipid (subtidal) and 114 μg g^{-1} lipid (intertidal). These values correspond to 2.8, 4.8 and 9.7 μg g^{-1} dry wt., respectively. (Halldórsson et al., 2005; Skarphéðinsdóttir et al., 2005).

Blue mussels (45–50 mm) were collected at low tide from the lower intertidal and transported moist and chilled to the laboratory in Sandgerði (<1 h transport time). Ten individuals from each site were immediately taken for evaluation of DNA single strand breaks, and the remaining mussels were placed in acclimation tanks upon arrival. The acclimation was held in running seawater (9.5 °C±0.3; salinity 32±1) originating from a bore hole extending 50 m into the ground. The seawater, flowing through the porous rock (lava), is free from particles and anthropogenic material and was aerated upon arrival to the building. The blue mussels were allowed to acclimatize for 4 days before the measurements.

2.2. Cardiograph

Cardiographic traces of heartbeat were obtained by means of a non-invasive method derived from Depledge and Andersen (1990). The sensor consisted of an Infra-Red light emitting diode axially coupled with a phototransistor. For cardiographic measurements, sensors were glued externally to the mussel shell in a position corresponding to the heart. The signals from the phototransistor were filtered and amplified with appropriate circuits, passed on to a Fluke 105B portable oscilloscope and automatically saved on a computer at programmed intervals. Recordings of heartbeat traces (duration 20 s, sampling frequency 12.5 Hz) were obtained for each mussel at set intervals (60, 120 and 180 min).

2.3. Feeding rate

Measurements of the feeding rate of the mussels (the volume of water cleared of algal cells per hour) were made using static system according to the procedure described by Widdows and Staff (1997) with minor modification. Each individual mussel was placed in a container holding 5 L of seawater with known algal concentration, and the exponential decline in cell concentration (>3 μm) was recorded.

An algal culture of *Isochrysis galbana* was added to each container reaching an initial concentration of ~25,000 cells mL^{-1}. After the addition of phytoplankton 3 min were allowed for the algal cells to be thoroughly mixed into the water column using magnetic stirrers. Care was taken not to disturb the mussels by placing the stirrers at the opposite side of the tanks which were kept in water bath to keep the temperature constant at 9.5 °C. A 20 mL sample from the water column of each tank was taken to measure its initial cell concentration. Later, six samples were taken from each tank at 30 min interval over a period of 3 h. The concentration of algal cells in each sample was measured using an electronic particle counter (Coulter Counter, model Z1) with a 100 μm orifice tube, counting in a 0.5 mL sub-sample each time. Three replicate counts were made on each sample and their mean calculated. Individual feeding rate was then estimated using the following equation:

$$\text{Feeding rate} = \text{Vol} * (\log C_1 - \log C_2)/\text{time}$$

where Vol is the volume of water in each tank, C_1 is the cell concentration at the start of each time increment, and C_2 is the cell concentration at the end of each time increment.