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.

2.4. Comet assay

The comet assay (single cell gel electrophoresis) was based on the protocol of Singh et al. (1988) with some modifications. Ten mussels (length 46±4 mm) from each group were used for analyses of DNA single strand breaks in haemocytes. Haemocytes were used because they are rapidly sampled, demanding short slide preparation time, and cell dissociation is not required, keeping possible cell damage to a minimum (Klobucar et al., 2003). Furthermore, haemocytes circulate in the open vascular system and thus, are closely exposed to environmental agents through transportation of toxicants and various defence mechanisms (Mersch et al., 1996). They are currently used for monitoring PAH exposure in the field (Thomas et al., 2007).

Hemolymph (~0.2 mL) was collected from the posterior adductor muscle, mixed with CMFS and centrifuged at 2000 rpm for 3 min. After discarding the supernatant, the pellet was resuspended in 0.5% low melting point agarose (LMP) and two 85 µl drops were placed on fully frosted slides, precoated with 0.75% normal melting point agarose. The samples were lysed for 2 h at 4 °C before they were randomly placed in the electrophoresis chamber and covered with chilled freshly made alkaline buffer (pH>13). The DNA was allowed to unwind for 20 min before electrophoresis (0.65 V/cm, 300 mA) for 30 min at 12 °C. All work was carried out in dim light to avoid UV damage to the DNA. As positive controls, four samples from Hvassahraun were exposed to 50 µM H_2O_2 for 1 h at 4 °C. The DNA was stained with 50 µl of ethidium bromide (20 µg ethidium bromide/mL distilled water) and examined with a fluorescence microscope (Leitz) coupled to a CCD camera and an image analysis package (Komet 5, Kinetic Imaging Ltd., Liverpool, U.K.). All slides were independently coded prior to examination and 100 (2×50) randomly selected cell nuclei were scored per slide. The number of DNA strand breaks are expressed as percentage of DNA in the tail (% Tail DNA). Highly damaged nuclei ("clouds") were excluded since they presumably represent dead cells (Hartmann and Speit, 1997).

2.5. Benzo[a]pyrene exposure

Benzo[a]pyrene (CAS: 50-32-8, cat. no: 159069, purity >99%) was purchased from ICN Biomedicals Inc. (Aurora, Ohio, USA). The mussels were exposed to the nominal dose of 100 µg B[a]P L^{-1} dissolved in 0.1% v/v acetone, or as controls to either 0.1% v/v acetone alone or clean seawater.

2.6. Experimental setup

On arrival to the laboratory blood samples were taken from 10 blue mussels from each site for evaluation of the background levels of DNA single strand breaks with the comet assay. Other mussels to be used in the experiments were placed in acclimation tanks, without feeding.

Prior to the start of the first experiment the blue mussels were equipped with the IR sensors and individually positioned in 5 L acclimation tanks. After 60 min, cardiac activity of each mussel (three consecutive traces of 20 s each) was recorded, and the specimens then randomly assigned to control or experimental groups. In the first experiment 10 individuals from the clean site Hvassahraun were submerged in the two different solutions; clean water as control and solution of clean water and 0.1% v/v acetone, and their cardiac activity (three consecutive traces of 20 s each) was individually recorded after 60, 120 and 180 min.

In the second experiment 40 individuals were similarly maintained in the control or test solutions (n=10 for each site and solution; 100 µg B[a]P L^{-1} and 0.1% v/v acetone) for 3 h. After the addition of phytoplankton three minutes were allowed for the algal cells to be thoroughly mixed into the water column and a 20 mL sample was taken from each tank to measure its initial cell concentration. Additionally six samples were taken from the water column of each tank at 30 min intervals over a period of 3 h for feeding rate measurements. The cardiac activity of the mussels was recorded (three consecutive traces of 20 s each) after 60, 120 and 180 min.

After the last measurement of the cardiac activity, the blue mussels were removed from the containers and blood extracted from the posterior adductor muscle of each mussel and prepared for the comet assay (described above).

2.7. Data elaboration and statistical analysis

All data from the experiments were analysed by means of two factor ANOVA, with origin and treatments as fixed factors. Data were checked for normality, and homogeneity of variances was tested with Cochran's test. Post hoc comparisons were performed with the Student-Newman-Keuls test. No transformations were needed for the heart rate and feeding rate data. The comet assay data (mean % tail DNA) were log transformed to achieve normality and homogeneity of variances and one-way ANOVA was used to compare all group means (field and experiments). The level of significance was set as $p<0.05$.

3. Results

3.1. Cardiac activity

The heart rate and consequently the metabolism of the mussels from the two sites differed considerably after the acclimatization period, before the addition of B[a]P (Fig. 1). Mussels from the clean site had lower heart rate (0.29 beats s^{-1}) than those from the polluted site (0.35 beats s^{-1}). These differences were highly significant (Table 1).

Secondly, the individuals from the two sites responded differently to the presence of B[a]P. The heart rate of the mussels from the clean site (Hvassahraun) did not change when exposed to the pollutant, while the specimens from the polluted site (Reykjavík harbor) responded significantly to B[a]P by increasing their heart rate (Fig. 1).

3.2. Feeding rate

Feeding rates (clearance of algal cells) were standardized to 1 g dry weight of soft tissue. Mussels from the clean site (Hvassahraun) had higher feeding rate (4.8 L h^{-1} g^{-1}) than the mussels from the polluted site (Reykjavík harbour; 1.6 L h^{-1} g^{-1}) and significant differences were explained only by the origin of the molluscs (Fig. 2 and Table 1).

The mussels from the clean site apparently showed a small decline in the feeding rate when exposed to B[a]P but it was 7% lower than for the control mussels of the same origin. On the contrary, mussels from Reykjavík harbour had, in presence of the toxicant, feeding rate 30% higher than the control mussels from the same site. These differences were, however, not significant when compared within sites.

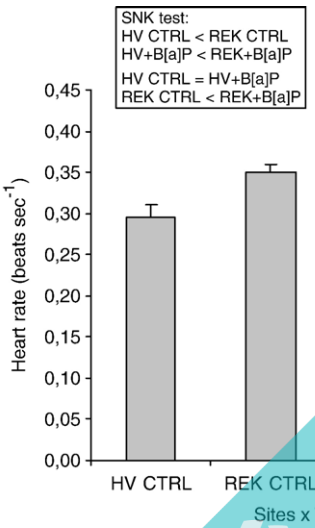

Fig. 1. Heart rate of the blue mussel *(Mytilus edulis)* during 3 h exposure to B[a]P. Values are expressed as beats s^{-1} (mean±SD, n=10). HV=Hvassahraun clean site; REK=Reykjavík harbour; CTRL=0.1% v/v acetone; B[a]P =100 µg benzo[a]pyrene L^{-1}+0.1% v/v acetone.

Table 1
Analysis of variance on the a) heart rate, b) feeding rate and c) DNA single strand break [$\log_e(x)$] results of the blue mussel (*Mytilus edulis*; $n=10$) from Hvassahraun (HV) and Reykjavík harbour (REK), with and without B[a]P treatment after 4 days of acclimatization

Source	df	MS	*F*	*P*
a) Heart rate				
Origin	1	0.065	40.39	***
Treatment	1	0.005	3.26	ns
Origin x Treatment	1	0.007	4.48	*
Residuals	36	0.002		
b) Feeding rate				
Origin	1	776.46	26.15	***
Treatment	1	0.05	0.02	ns
Origin x Treatment	1	18.45	0.62	ns
Residuals	36	29.69		
c) DNA single strand breaks				
Origin	1	0.014	0.085	ns
Treatment	1	0.238	1.404	ns
Origin x Treatment	1	0.229	1.349	ns
Residuals	3996	0.170		
Field vs. experiment				
Groups	5	1.492	8.432	***
Residuals	5994	118.2		

Post hoc comparisons were performed with the Student-Newman-Keuls test. *=$p<0.05$; ***=$p<0.001$; ns=not significant.

3.3. Comet assay

The amount of DNA single strand breaks (mean % tail DNA) in haemocytes was significantly higher in mussels from the polluted site (Reykjavík harbour; 12.26% tail DNA) compared to the mussels from the clean site (Hvassahraun; 9.65% tail DNA) when measured directly from the field (Fig. 3 and Table 1). After 4 days of acclimatization, and at the end of the experiment, the amount of DNA strand breaks was similar for both populations/treatments and similar to the initial level at the clean site.

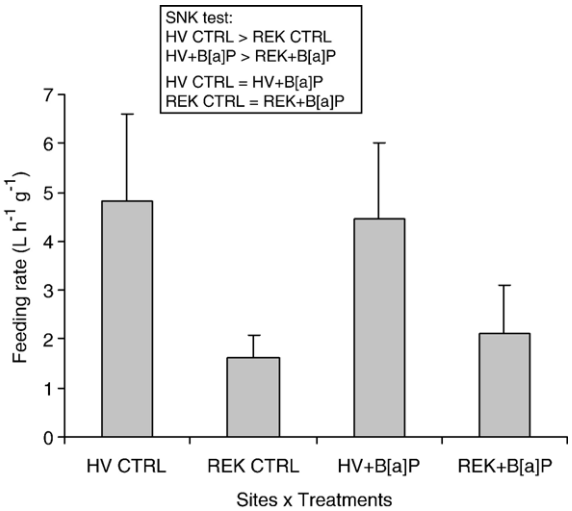

Fig. 2. Feeding rate of the blue mussel (*Mytilus edulis*) during 3 h exposure to B[a]P. Values are in L h^{-1} g^{-1} (mean±SD, $n=10$). HV=Hvassahraun clean site; REK=Reykjavík harbour; CTRL=0.1% v/v acetone; B[a]P=100 µg benzo[a]pyrene L^{-1}+0.1% v/v acetone.

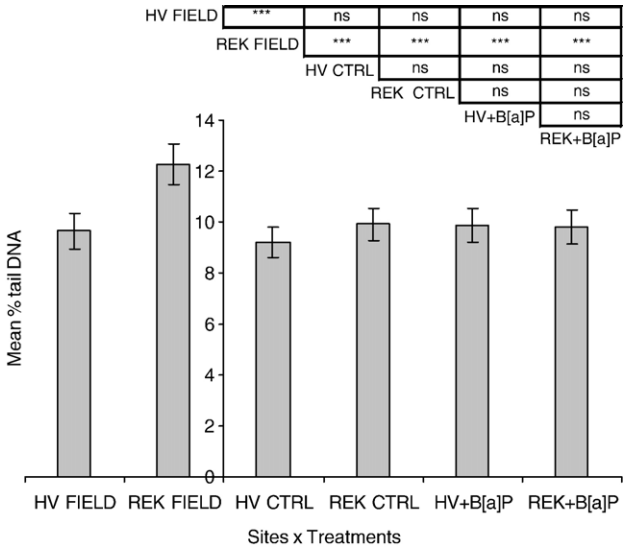

Fig. 3. DNA single strand breaks in haemocytes of the blue mussel (*Mytilus edulis*) measured before, and after acclimatization and 3 h exposure to B[a]P. Values are expressed as mean % tail DNA (mean±95% C.I., $n=10$). HV=Hvassahraun clean site; REK=Reykjavík harbour; FIELD=No laboratory acclimatization before measurements; CTRL=0.1% v/v acetone; B[a]P=100 µg benzo[a]pyrene L^{-1}+0.1% v/v acetone. SNK post hoc comparison between all samples. ***=$p<0.001$; ns=not significant.

4. Discussion

The clear difference in background pollution, as well as the short distance (~20 km) between the sites, provided the basis for comparing immediate responses of natural mussel populations to a PAH compound. The mussels from Reykjavík harbour were apparently under considerable stress, observed both at the cellular level (DNA damages) and physiological level (feeding rate and heart rate). The elevated levels of DNA single strand breaks in mussels sampled directly from the field indicate recent oxidative damages, presumably caused by PAHs and other toxic hydrocarbons in the harbour. In concordance, previous studies on mussels from the same sites revealed significant pollution (PAHs and Organotins) in Reykjavík harbour (Ericson et al., 2002; Halldórsson et al., 2004, 2005; Skarphéðinsdóttir et al., 2005; Da Ros et al., 2007). After 4 days in clean seawater the amount of detectable strand breaks had reduced and was similar as in mussels from the clean site. Although DNA repair can occur in matter of hours in bivalves (Pruski and Dixon, 2002; Villela et al., 2006), the recovery we observed could be explained by the repair of DNA damages and/or by replacement of damaged cells through apoptosis or cell death (Pruski and Dixon, 2002; Hook and Lee, 2004). These results support previous findings on rapid recovery of DNA strand breaks when organisms are allowed to acclimatize in a clean environment, stressing the need for rapid sampling when used in field monitoring. Increased heart rates and lowered feeding rates were still observed after the acclimation period for the harbour

mussels, suggesting ongoing removal of toxicants from the body.

The short time exposure to the nominal dose of 100 μg B[a]P L^{-1} did not exert DNA single strand breaks in haemocytes or lead to significant changes in the feeding rate of mussels from either site, although noteworthy trends in feeding rates were observed. The feeding rate of the mussels from the clean site decreased by 7%, while the mussels from the contaminated site showed about 30% increase in feeding rate in presence of the pollutant, suggesting elevated physiological activity. The most noticeable difference between the two sites was, however, seen in the cardiac activity of the blue mussels. The heart rate of the mussels from the clean site did not change when exposed to the high B[a]P concentration, while mussels from the contaminated site increased their heart rate significantly when exposed to B[a]P.

The accumulation rate of B[a]P by the blue mussel can be very rapid. Blue mussels exposed to 1 ppb B[a]P for 6 h, followed by 6 h in clean seawater, had tissue concentrations 70–80% of the initial B[a]P concentration in the water (Durand et al., 2002). Several studies have also shown acute effects of B[a]P on the DNA integrity of bivalves (Bihari and Fafandel, 2004; Siu et al., 2004; Kim and Hyun, 2006). These effects are mainly through metabolism of B[a]P to reactive intermediates (e.g. epoxides, quinones, cation radicals) leading to oxidative lesions (Mitchelmore et al., 1998). Siu et al. (2004) observed for example significantly increased DNA strand breaks in haemocytes of the green-lipped mussel (*Perna viridis*) after 1 day exposure to 30 μg B[a]P L^{-1} (measured by means of the comet assay). The exposure to B[a]P in the present study did, however, not generate DNA strand breaks in haemocytes despite the high nominal concentration. The exposure time was shorter in this experiment (3 h) compared to other short-term *in vivo* genotoxical studies using B[a]P (≥24 h), suggesting longer exposure time needed to exert genotoxic effects.

Reduced scope for growth of the blue mussel has been related to PAH exposure in the field, primarily due to reduction in feeding rate (i.e. clearance rate) (Widdows et al., 1997; Halldórsson et al., 2005). PAHs can exert narcotic effects on the gill ciliary activity, leading to decreased feeding rate, although many of the high molecular weight PAHs do not exert direct toxic effects (Donkin et al., 1989, 1991). However, the high molecular weight B[a]P has been shown to induce blood cell lysosomal membrane damages in the blue mussel, possibly due to direct toxic effect of B[a]P (Okay et al., 2000). Only minor effects were observed on the feeding rate in the present study, not indicating immediate toxic effects on the gill ciliary activity.

This study revealed an interesting difference in the heart rate response, depending on the background of the organisms. Firstly, mussels coming from the polluted site had higher baseline heart rates relative to the ones from the clean site. Similar results were found for other molluscan species and in a different ecological context (Nicholson, 1999; Wedderburn et al., 2000; Chelazzi et al., 2004). Secondly, when exposed to B[a]P the heart rate of the polluted mussels increased significantly while no effects were seen in mussels from the clean site. Little information is available on the effects of PAHs on the heart rate of other marine molluscs. However, Galloway et al. (2002) found strong correlation between increased heart rates of the ribbed mussel (*Geukensia demmissa*) and PAHs and PCBs in a field study. Effects of copper on the heart rate of the blue mussel are well documented but opposite to our findings the heart rate generally decreases in mussels exposed to unrealistically high levels of copper (e.g. Grace and Gainey, 1987; Curtis et al., 2000, 2001; Brown et al., 2004). Copper has been suggested to stimulate an inhibitory cholinergic neuronal pathway to the heart in the blue mussel, leading to reduced heart rate (Curtis et al., 2001). However, elevated heart rate at high copper levels has been observed for the green-lipped mussel (*P. viridis*) and increased heart rate of this species when transplanted to polluted coastal sites in Hong Kong was suggested to reflect enhanced metabolism during detoxification process (Nicholson, 2003; Nicholson and Lam, 2005).

In the present study the higher baseline heart rate of the blue mussels collected from the polluted site suggests ongoing detoxification process, but the fact that only these mussels responded to B[a]P does not indicate immediate toxic effects, but rather adaptive response to the contaminant, i.e. reflecting the pre-exposure history. The lack of heart rate response of the mussels from the clean site indicates a limited sensitivity of this biomarker, demonstrating that further studies are required for its application to pollution monitoring at high-latitudes. Although all the biomarkers revealed a significant difference between the study sites, the results from the laboratory experiments indicate limited sensitivity and temporal predictivity of these non-specific biomarkers. Thus, further investigation on the temporal predictivity, i.e. transient measurable changes of the heart rate and DNA strand breaks in haemocytes, is suggested. The ability of the mussels to modulate metabolism depending on the environmental background pollution demonstrates as well that the background of the organisms should be considered when evaluating short-term biomarker responses to contaminants.

Acknowledgements

This study was supported by the European Commission—Access to Research Infrastructures, action of the Improving Human Potential Programme (IHP). We thank the anonymous reviewers for their helpful comments.

References

ACIA (Arctic Climate Impact Assessment). Impacts of a warming Arctic. Cambridge University Press; 2004. Available from: http://www.acia.uaf.edu.

ACIA (Arctic Climate Impact Assessment). Cambridge University Press; 2005. p. 1042. Available from: http://www.acia.uaf.edu.

Albaigés J, Morales-Nin B, Vilas F. The Prestige oil spill: a scientific response. Mar Pollut Bull 2006;53:205–7.

AMAP (Arctic Monitoring and Assessment Programme). Arctic Pollution 2002. Oslo: Norway; 2002. p. 112. Vii + Available from: http://www.amap.no.

Anderson JW, Lee RF. Use of biomarkers in oil spill risk assessment in the marine environment. Hum Ecol Risk Assess 2006;12:1192–222.
Bihari N, Fafandel M. Interspecies differences in DNA single strand breaks caused by benzo(a)pyrene and marine environment. Mutat Res 2004;552: 209–17.
Bolognesi C, Frenzilli G, Lasagna C, Perrone E, Roggieri P. Genotoxicity biomarkers in *Mytilus galloprovincialis*: wild versus caged mussels. Mutat Res 2004;552:153–62.
Bowen RE, Depledge MH. Rapid assessment of marine pollution (RAMP). Mar Pollut Bull 2006;53:631–9.
Brown RJ, Galloway TS, Lowe D, Browne MA, Dissanayake A, Jones MB, et al. Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. Aquat Toxicol 2004;66:267–78.
Chelazzi G, De Pirro M, Williams GA. Different cardiac responses to copper in limpets from metal polluted and clean shores of Hong Kong. Mar Environ Res 2004;58:83–93.
Courtenay SC, Grunwald CM, Kreamer GL, Fairchild WL, Arsenault JT, Ikonomou M, et al. A comparison of the dose and time response of CYP1A1 mRNA induction in chemically treated Atlantic tomcod from two populations. Aquat Toxicol 1999;47:43–69.
Curtis TM, Williamson R, Depledge MH. Simultaneous, long-term monitoring of valve and cardiac activity in the blue mussel *Mytilus edulis* exposed to copper. Mar Biol 2000;136:837–46.
Curtis TM, Williamson R, Depledge MH. The initial mode of action of copper on the cardiac physiology of the blue mussel, *Mytilus edulis*. Aquat Toxicol 2001;52:29–38.
Da Ros L, Moschino V, Guerzoni S, Halldórsson HP. Lysosomal responses and metallothionein induction in the blue mussel *Mytilus edulis* from the south–west coast of Iceland. Environ Int 2007;33:362–9.
De Pirro M, Marshall DJ. Phylogenetic differences in cardiac activity, metal accumulation and mortality of limpets exposed to copper: a prosobranch-pulmonate comparison. J Exp Mar Biol Ecol 2005;322:29–37.
Depledge MH, Andersen BB. A computer-aided physiological monitoring system for continuous, long-term recording of cardiac activity in selected invertebrates. Comp Biochem Physiol A 1990;96:474–7.
Donkin P, Widdows J, Evans SV, Worrall CM, Carr M. Quantitative structure-activity relationships for the effect of hydrophobic organic chemicals on rate of feeding by mussels (*Mytilus edulis*). Aquat Toxicol 1989;14:277–93.
Donkin P, Widdows J, Evans SV, Brinsley MD. QSARs for the sublethal responses of marine mussels (*Mytilus edulis*). Sci Total Environ 1991;109–110:461–76.
Durand F, Peters LD, Livingstone DR. Effect of intertidal compared to subtidal exposure on the uptake, loss and oxidative toxicity of water-born benzo[a]pyrene in the mantle and whole tissues of the mussel, *Mytilus edulis* L. Mar Environ Res 2002;54:271–4.
Eertman RHM, Groenink CLFMG, Sandee B, Hummel H, Smaal AC. Response of the blue mussel *Mytilus edulis* L. following exposure to PAHs or contaminated sediment. Mar Environ Res 1995;39:169–73.
Emmanouil C, Green RM, Willey FR, Chipman JK. Oxidative damage in gill of *Mytilus edulis* from Merseyside, UK, and reversibility after depuration. Environ Pollut in press. doi:10.1016/j.envpol.2007.03.013.
Ericson G, Skarphéðinsdóttir H, Zuanna LD, Svavarsson J. DNA adducts as indicators of genotoxic exposure in indigenous and transplanted mussels, *Mytilus edulis*, L. from Icelandic coastal sites. Mutat Res Genet Toxicol Environ 2002;516:91–9.
Galloway TS. Biomarkers in environmental and human health risk assessment. Mar Pollut Bull 2006;53:606–13.
Galloway TS, Sanger RC, Smith KL, Fillmann G, Readman JW, Ford TE, et al. Rapid assessment of marine pollution using multiple biomarkers and chemical immunoassays. Environ Sci Technol 2002;36:2219–26.
Galloway TS, Brown RJ, Browne MA, Dissanayake A, Lowe D, Jones MB, et al. Ecosystem management bioindicators: the ECOMAN project—a multi-biomarker approach to ecosystem management. Mar Environ Res 2004;58:233–7.
Garcia MP, Livingstone DR. Benzo[a]pyrene-dione stimulated oxyradical production by microsomes of digestive gland of the common mussel, *Mytilus edulis* L. Mar Environ Res 1995;39:185–9.
Grace AL, Gainey LF. The effects of copper on the heart rate and filtration rate of *Mytilus edulis*. Mar Pollut Bull 1987;18:87–91.
Halldórsson HP, Ericson G, Svavarsson J. DNA strand breakage in mussels (*Mytilus edulis* L.) deployed in intertidal and subtidal zone in Reykjavík harbour. Mar Environ Res 2004;58:763–7.
Halldórsson HP, Svavarsson J, Granmo Å. The effect of pollution on scope for growth of the mussel (*Mytilus edulis* L.) in Iceland. Mar Environ Res 2005;59:47–64.
Hartmann A, Speit G. The contribution of cytotoxicity to DNA-effects in the single cell gel test (comet assay). Toxicol Lett 1997;90:183–8.
Hebel DK, Jones MB, Depledge MH. Responses of crustaceans to contaminant exposure: a holistic approach. Estuar Coast Shelf Sci 1997;44:177–84.
Hook SE, Lee RF. Genotoxicant induced DNA damage and repair in early and late developmental stages of the grass shrimp *Paleomonetes pugio* embryo as measured by the comet assay. Aquat Toxicol 2004;66:1–14.
IARC (International Agency for Research on Cancer). Overall evaluations of carcinogenicity. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Supplement 7. France: Lyon; 1987. p. 440.
Kim IY, Hyun CK. Comparative evaluation of the alkaline comet assay with the micronucleus test for genotoxicity monitoring using aquatic organisms. Ecotoxicol Environ Saf 2006;64:288–97.
Klobucar GIV, Pavlica M, Erben R, Papes D. Application of the micronucleus and comet assays to mussel *Dreissena polymorpha* haemocytes for genotoxicity monitoring of freshwater environments. Aquat Toxicol 2003;64: 15–23.
Large AT, Shaw JP, Peters LD, McIntosh AD, Webster L, Mally A, et al. Different levels of mussel (*Mytilus edulis*) DNA strand breaks following chronic field and acute laboratory exposure to polycyclic aromatic hydrocarbons. Mar Environ Res 2002;54:493–7.
Livingstone DR, Moore MN, Lowe DM, Nasci C, Farrar SV. Responses of the cytochrome *P*-450 monooxygenase system to diesel oil in the common mussel, *Mytilus edulis* L., and the periwinkle, *Littorina littorea* L. Aquat Toxicol 1985;7:79–91.
Loureiro ML, Ribas A, López E, Elena O. Estimated costs and admissible claims linked to the Prestige oil spill. Ecol Econ 2006;59:48–63.
Lowe DM, Moore MN, Readman JW. Pathological reactions and recovery of hepatopancreatic digestive cells from the marine snail *Littorina littorea* following exposure to a polycyclic aromatic hydrocarbon. Mar Environ Res 2006;61:457–70.
Ma XL, Cowles DL, Carter RL. Effect of pollution on genetic diversity in the bay mussel *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*. Mar Environ Res 2000;50:559–63.
Mersch J, Beauvais MN, Nagel P. Induction of micronuclei in haemocytes and gill cells of zebra mussels, *Dreissena polymorpha*, exposed to clastogens. Mutat Res 1996;371:47–55.
Mitchelmore CL, Birmelin C, Chipman JK, Livingstone DR. Evidence for cytochrome P-450 catalysis and free radical involvement in the production of DNA strand breaks by benzo[a]pyrene and nitroaromatics in mussel (*Mytilus edulis* L.) digestive gland cells. Aquat Toxicol 1998;41: 193–212.
Moore MN, Allen JI, McVeigh A. Environmental prognostics: an integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. Mar Environ Res 2006;61:278–304.
Nicholson S. Cardiac and lysosomal responses to periodic copper in the mussel *Perna viridis* (Bivalvia: Mytilidae). Mar Pollut Bull 1999;38:1157–62.
Nicholson S. Cardiac and branchial physiology associated with copper accumulation and detoxication in the mytilid mussel *Perna viridis* (L.). J Exp Mar Biol Ecol 2003;295:157–71.
Nicholson S, Lam PKS. Pollution monitoring in Southeast Asia using biomarkers in the mytilid mussel *Perna viridis* (Mytilidae: Bivalvia): review article. Environ Int 2005;31:121–32.
Okay OS, Donkin P, Peters LD, Livingstone DR. The role of algae (*Isochrysis galbana*) enrichment on the bioaccumulation of benzo[a]pyrene and its effects on the blue mussel *Mytilus edulis*. Environ Pollut 2000;110:103–13.
Pruski AM, Dixon DR. Effects of cadmium on nuclear integrity and DNA repair efficiency in the gill cells of *Mytilus edulis* L. Aquat Toxicol 2002;57: 127–37.
Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of damage in individual cells. Exp Cell Res 1988;175:184–91.

Siu WHL, Cao J, Jack RW, Wu RSS, Richardson BJ, Xu L, et al. Application of the comet and micronucleus assays to the detection of B[a]P genotoxicity in haemocytes of the green-lipped mussel (*Perna viridis*). Aquat Toxicol 2004;66:381–92.

Skarphéðinsdóttir H, Ericson G, Zuanna LD, Gilek M. Tissue differences, dose–response relationship and persistence of DNA adducts in blue mussels (*Mytilus edulis* L.) exposed to benzo[a]pyrene. Aquat Toxicol 2003;62: 165–77.

Skarphéðinsdóttir H, Ericson G, Halldórsson HP, Svavarsson J. Seasonal and intertidal impact on DNA adduct levels in gills of blue mussels (*Mytilus edulis* L.). Environ Pollut 2005;136:1–9.

Skarphéðinsdóttir H, Ericson G, Svavarsson J, Næs K. DNA adducts and polycyclic aromatic hydrocarbon (PAH) tissue burden in mussels (*Mytilus edulis* L.) from Nordic coastal sites. Mar Environ Res 2007;64:479–91.

Thomas RE, Lindberg M, Harris PM, Rice SD. Induction of DNA strand breaks in the mussel (*Mytilus trossulus*) and clam (*Protothaca staminea*) following chronic field exposure to polycyclic aromatic hydrocarbons from the Exxon Valdez spill. Mar Pollut Bull 2007;54:726–32.

UNEP. Pedersen SA, Madsen J, Dyhr-Nielsen M. Arctic Greenland, East Greenland Shelf, West Greenland Shelf, GIWA Regional assessment 1b, 15, 16. Kalmar, Sweden: University of Kalmar; 2004.

Villela IV, de Oliveira IM, da Silva J, Henriques JAP. DNA damage and repair in haemolymph cells of golden mussel (*Limnoperna fortunei*) exposed to environmental contaminants. Mutat Res 2006;605:78–86.

Wedderburn J, McFadzen I, Sanger RC, Beesley A, Heath C, Hornsby M, et al. The field application of cellular and physiological biomarkers, in the mussel *Mytilus edulis*, in conjunction with early life stage bioassays and adult histopathology. Mar Pollut Bull 2000;40:257–67.

Widdows J, Staff F. Practical procedures for the measurement of scope for growth. Plymouth, U.K.: Plymouth Marine Laboratory, Prospect Place, West Hoe; 1997.

Widdows J, Nasci C, Fossato VU. Effects of pollution on the scope for growth of mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy. Mar Environ Res 1997;43:69–79.

Yngvadóttir E, Mikaelsdóttir A, Jónsdóttir I, Ragnarsdóttir Þ. Monitoring of the marine biosphere around Iceland 2004 and 2005. Icelandic Fisheries Laboratories project report 10-06. Reykjavík, Iceland: Ministry for the Environment; 2006.