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Tissue-specific differences in transcriptomic and genotoxic responses of marine mussels exposed to benzo-a-pyrene

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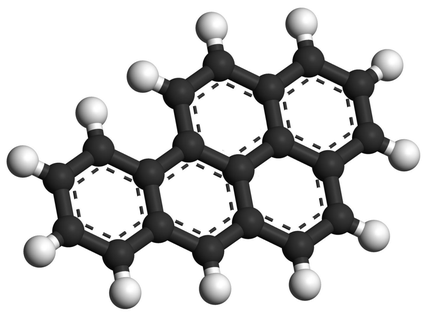
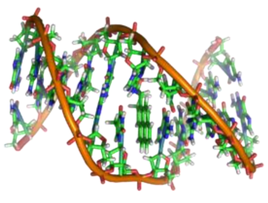
Introduction

Benzo(a)pyrene (BaP) is a ubiquitous polycyclic aromatic hydrocarbon, found in cigarette smoke and diesel exhaust as well as many industrial sources.



BaP is a pro-carcinogen and has well-established toxic effects in both human and non-human biota. In particular, its metabolites (especially benzo[a]pyrene diol epoxide [BPDE]) form adducts with DNA.

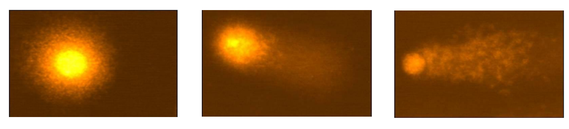


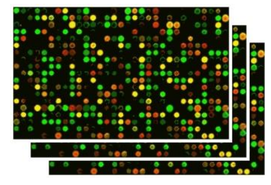
BaP has been shown to induce genotoxic effects, including DNA damage and DNA adducts, in marine mussels. It also induces lysosomal damage and other toxic effects. However, the adverse outcome pathways that lead to these effects are not as well elucidated in mussels as they are for mammalian model species.

Bivalve molluscs, including mussels (*Mytilus galloprovincialis*), are sessile, filter feeders with an important ecological role in many coastal ecosystems. As such, they are an important bioindicator species.

Aims:

- Validate a new low-density mussel microarray, under controlled laboratory conditions (for more details see **PL-097**).
  - Contains 476 probes
  - All genes putatively identified and associated with 16 biological processes, including DNA repair
- Combine established biomarkers of genotoxicity with an 'omics approach in order to reveal potential mechanistic causes of BaP-induced effects in mussels.





Methods

Mussels were exposed to BaP for 3d (0.5 mussels L^{-1}), using a static exposure with no renewal of water or contaminant. Mussels were not fed.

***n* = 9**

- Vehicle control (0.02 % DMSO)
- 5 µg L^{-1} BaP
- 50 µg L^{-1} BaP
- 500 µg L^{-1} BaP
- 1000 µg L^{-1} BaP

After 3d exposure, mussel gills and digestive gland were sampled for:

- Comet assay (DNA damage)
- P-32 postlabelling (DNA adducts)
- Hybridisation of cDNA to a new targeted low density microarray (for more details see **PL-097**)
- Measurement of BaP in water and tissues (GC-MS)

Mussels at the highest concentration were sampled for DNA adducts only

Figure 1. Schematic illustration of experimental design.

Results

- BaP concentrations in water remained stable over the first hour of exposure, but declined rapidly after 24h (Figure 2). This is likely due to adsorption (onto glass or mussels) and removal by the mussels.
- Concentrations of BaP in the mussels' digestive glands increased with exposure concentration, as expected, and peaked at 137.7 µg/g (Table 1).
- As expected, BaP induced genotoxic effects in mussels, with significant increases in % tail DNA for both tissues (Fig. 3).
- There were, however, differences between the tissues, with digestive gland showing higher levels of DNA damage than gill (Fig. 3).
- Initial analysis has detected adducts (dG-N2-BPDE) in the digestive gland only (Fig. 4a).
- Female mussels had higher levels of DNA adducts at both concentrations tested so far (Fig. 4b). Further analysis is being completed to confirm if this is a concentration-dependent effect.
- DNA metabolism genes that were differentially expressed are shown in Table 2.

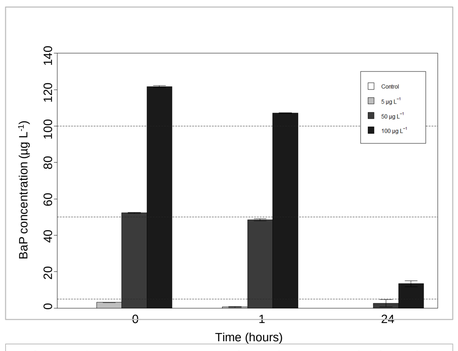


Figure 2. BaP concentrations in water during the first 24 h of 3 d exposure.

Table 1. Indicative values for BaP concentration in mussel digestive gland, after 3d exposure to BaP.

Treatment	Control	5 µg L^{-1}	50 µg L^{-1}	100 µg L^{-1}
DG dry weight (mg)	52	68	70	55
DG water content (% of total weight)	76	76	76	75
DG lipid content (mg/g dry weight)	52	74	154	135
DG BaP content (µg/g dry weight)	< 0.5	6.4	58.3	137.7

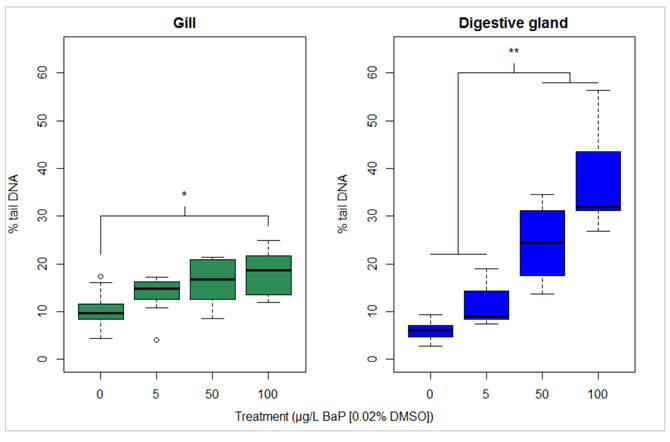


Figure 3. DNA damage (as measured by comet assay) in mussel gill and digestive gland cells after 3d exposure to BaP.

Table 2. Fold change of differentially expressed DNA metabolism genes in mussels after BaP exposure, as determined by microarray hybridisation.

		Gills			Digestive gland	
Gene ID	Gene Name	5 µg L^{-1}	50 µg L^{-1}	100 µg L^{-1}	50 µg L^{-1}	100 µg L^{-1}
HQ424449.1	caspase 2 mRNA		1.456			
HQ424450.1	caspase 8 mRNA		1.548		1.938	
KC545831.1	Bax inhibitor-1 protein (BI1)		1.51	1.483		
AJ516519.1	adp-ribosylation factor 1		1.289	-0.997	-2.091	
AJ625243.1	delta-n p63 p73-like protein		-1.607			
AJ625979.1	defender against cell death 1		1.55		-1.113	
AJ625479.1	guanine nucleotide binding2	-3.771	-2.212	-4.403		-1.576
AJ516663.1	h2a histonemember z		-2.048			
AF227976.1	*Mytilus edulis* topoisomerase II mRNA			-1.54		
AJ624686.1	dna ligase i			-2.274		-2.451
AJ516600.1	h3family 3b			1.257		
AJ626239.1	member ras oncogene family			1.363		1.539
DQ865150.1	p63/73-like protein				-1.289	
DQ060436.1	delta-N p63/p73-like protein mRNA				-1.527	
AJ623737.1	*Mytilus galloprovincialis* haemolymph, cDNA clone gadd45a				1.797	
AY579472.1	*Mytilus edulis* p53 tumor suppressor-like protein mRNA				-2.578	
AJ625027.1	member ras oncogene family				1.585	
HQ424454.1	caspase 3/7-4 mRNA					1.549
AY679522.1	*Mytilus edulis* RAS mRNA					1.485
AJ625058.1	t-cell lymphoma invasion and metastasis 1					-1.782

Heat map colours indicate degree of fold change (whether negative or positive).

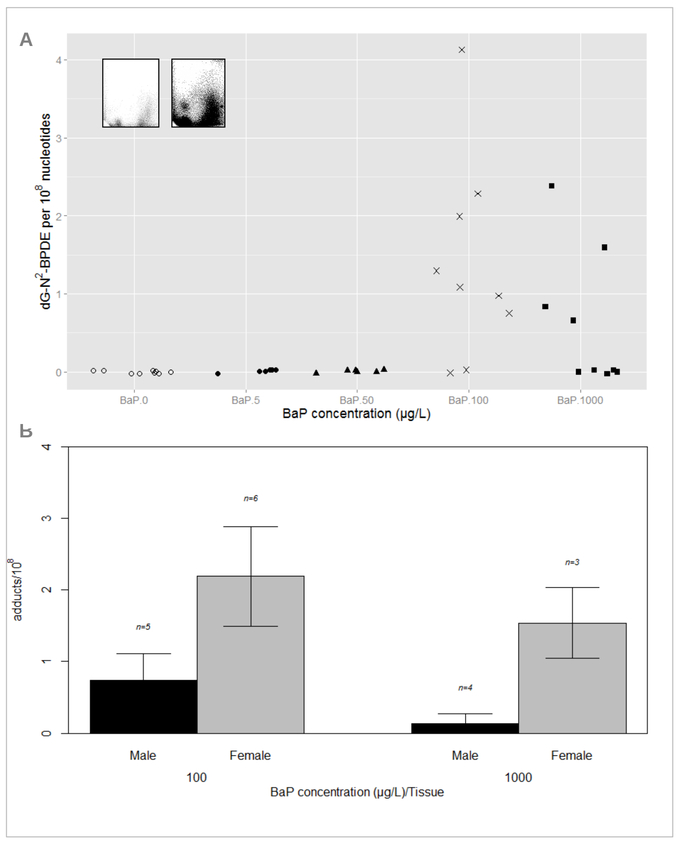


Figure 4. DNA adducts (a) digestive gland at all BaP concentrations, including representative examples of absent and present adducts (L to R); (b) sex-specific differences at higher concentrations.