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**DNA ADDUCTS AND POLYCYCLIC AROMATIC HYDROCARBON
(PAH) TISSUE LEVELS IN BLUE MUSSELS (*MYTILUS SPP.*) FROM
NORDIC COASTAL SITES**

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ABSTRACT

DNA adducts in gills and digestive gland, as well as polycyclic aromatic hydrocarbon (PAH) tissue levels were analysed in blue mussels (*Mytilus* spp.) from Nordic coastal areas (Iceland, Norway and Sweden) with diffuse or point sources of PAHs of various origins. Both DNA adduct and PAH tissue levels were generally low, indicating low PAH exposure to the mussels in the areas studied. DNA adducts were found to be higher in gills than in digestive gland of the mussels at all sites studied. Elevated DNA adduct levels in gills were found at 6 sites out of 18 compared to reference sites in respective coastal zones. Adduct levels ranged from 0.5 - 10 nmol adducts/mol normal nucleotides, being highest in mussels from Reykjavík harbour, Iceland (intertidal mussels), and from Fiskaatangen, Norway (subtidal mussels). Total PAH tissue levels in the mussels ranged between 40 – 11670 ng/g dry wt., and were significantly correlated with DNA adduct levels ($r^2=0.73$ $p<0.001$). PAH ratio values indicated that the PAHs were in most cases of pyrolytic origin, but with petrogenic input near harbours and an oil refinery.

Keywords: DNA adducts, Genotoxicity, Biomarker, Polycyclic aromatic hydrocarbons, *Mytilus* spp., Field study, Nordic coastal waters

INTRODUCTION

Among the variety of natural and anthropogenic contaminants in the aquatic environment there are many that are genotoxic, i.e. capable of interacting with the genetic material. For example, polycyclic aromatic hydrocarbons (PAHs) have well documented toxic, mutagenic and carcinogenic properties, which along with the fact that they are widely distributed in the environment, makes them priority pollutants (Neff, 1979). PAHs derive from incomplete combustion of fossil fuels, creosote production, discharges from electrochemical industry such as aluminium smelters, and oil spills (Lorenz and Glovik, 1972; Neff, 1979).

PAHs are for example known to cause genetic toxicity through the formation of DNA adducts. DNA adducts are produced by the formation of covalent bonds between a genotoxic chemical or its metabolite and the DNA molecule (Miller & Miller, 1981). Damaged DNA that is not repaired in time may have direct consequences like cell death, tumour initiation, embryo toxicity, mutations or other dysfunctions (Shugart, 1995; Kurelec, 1993). Analysis of DNA adducts by the ^{32}P -postlabelling method has in many field studies on fish been confirmed as a sensitive and reliable biomarker of exposure to and effects of PAHs (e.g. Stein et al., 1993; Reichert et al., 1998; Ericsson et al., 1999). DNA adduct formation and analysis in marine Mytilid bivalve mussels has recently received increasing attention. Mussels have less active enzyme system than fish for biotransformation of xenobiotics, but they do possess the metabolic capacity to activate potentially genotoxic compounds to reactive intermediates capable of binding to DNA (e.g. Livingstone et al., 1984; Harvey and Parry, 1997). Experimental studies have shown that Mytilid mussels form DNA adducts following exposure to PAHs, amines and nitro aromatic substances (e.g. Marsh et al., 1993; Venier and Canova, 1996; Harvey and Parry, 1997; 1998; Akcha et al., 2000; Skarphedinsdottir et al., 2003). Field studies on DNA adduct levels in Mytilids are few (Kurelec, 1990; Venier et al.,

1996; Sole et al., 1996; Harvey et al., 1997; Harvey et al., 1999; Ericson et al., 2002; Skarphedinsdottir et al., 2005), although mussels are important components of the coastal ecosystems and are in many ways ideal bioindicator organisms.

In this study the objective was to i) study and characterise DNA adduct levels in blue mussels (*Mytilus* spp.) as well as PAH mussel tissue levels from Nordic coastal waters with different PAH pollution loads, ii) to investigate which tissue, gills or digestive gland, had the highest adduct levels and iii) to see if DNA adduct values correlated with PAH tissue levels. The sites chosen for this study were sites suspected to have various PAH origins, mixtures and levels, such as harbours of different sizes, industrialised and urban sites, as well as unpolluted sites for reference.

MATERIALS AND METHODS

Chemicals

Salmon sperm DNA (S-1626), spermidine (S-2626), dithiothreitol (D-9779), RNase A (R-4642), micrococcal endonuclease (N-3755), spleen phosphodiesterase (P-9041) and polyethylenimine (P3143) were obtained from Sigma-Aldrich Sweden AB, Stockholm, Sweden. Proteinase K (1000144), α -amylase (102814), RNase T₁ (109 193), nuclease P1 (236 225), T4 polynucleotide kinase (3' phosphatase-free, 838 292) and phenol (1814303) were supplied by Roche Diagnostics Scandinavia AB, Sweden. [γ -³²P]ATP was obtained from Amersham Pharmacia Biotech (now General Electric healthcare), Uppsala, Sweden, and cellulose (MN 301) from Tamro MedLab AB, Mölndal, Sweden. The standard adduct 7R, 8S, 9S-trihydroxy, 10R- (N²-deoxyguanosyl-3'-phosphate)-7, 8, 9, 10-tetrahydro-benzo[a]pyrene

(BaPDE-dG-3'p) was obtained from NCI Chemical Repository, Midwest Research Institute, Kansas City, Missouri, USA. All other chemicals used were of analytical grade or higher.

Sampling sites

Blue mussels (*Mytilus* spp.) were collected from 22 different coastal sites in Iceland, Sweden and Norway in October 2000. The sampling sites are indicated in Figure 1 and described in Table 1. The sites chosen were harbours of different sizes and industrialised/urban sites with suspected or already documented PAH contamination in bottom sediments or organisms, and reference sites without any site-specific pollution.

In Sweden, blue mussels (*Mytilus* spp.) were collected at 5 sites along the east coast; the brackish Baltic Sea, and *Mytilus edulis* was sampled at 6 sites along the west coast; in Skagerrak. Askö represents a reference site in the Baltic Sea, and the site Fjällbacka, which has been used as a reference site in field investigations of pollutant levels and effects on fish, represents the reference site on the Swedish west coast. The other Swedish sites were chosen due to their close location to industries, i.e. Oxelösund (steel plant), Svartören and Svartlingskär (pulp industry), Stenungsund (petrochemical industry), Brofjorden (oil refinery) or heavily urbanised areas such as Göteborg (Gothenburg), where one of the sampling sites is located close to the mouth of the river Göta älv along which several industries and harbours are located. In Sweden the tidal water range is ~0.2m on the west coast, but on the east coast in the Baltic Sea, it is practically zero. Average sea temperature and salinity for October 2000 at the Swedish west coast sites were ~13°C, and ~24‰, and for the Baltic Sea sampling sites, 10-11°C, and 6-7‰ (Based on data provided by the Swedish Meteorological and Hydrological Institute (SMHI), Stockholm Marine Research Centre (SMF) and HavsFiskeLaboratoriet in the frame of the Swedish Environmental Monitoring Programme).

Along the Norwegian coast, blue mussels (*Mytilus edulis*) were collected at 4 different sites, i.e. at Croftholmen, Valløy, Fiskaatangen, and Sauda. Three of the stations (Croftholmen, Valløy, Fiskaatangen) are at the southern part of Norway along the Skagerrak Sea, while Sauda is located in an inner part of a fjord at the western coast of Norway. Croftholmen, Fiskaatangen, and Sauda represent areas with PAH point sources. Croftholmen is located in Norway's most industrialised area and influenced both by heavy merchant ship traffic and effluents from a manganese smelter. The same apply to the samples from Fiskaatangen (anode plant for manganese smelters and commercial harbour) and Sauda (manganese-alloy production). Valløy represents the reference site, it is located in open coastal waters with no specific point source. The tidal water range is ~0.6m at Croftholmen, Valløy and Fiskaatangen, and ~0.8m at Sauda. Sea temperature was measured at the sites and ranged from 12-14°C. Salinity at Croftholmen is in average ~25‰, and ~30-33 ‰ at Fiskaatangen, Sauda and Valløy (Based on data from the Norwegian Meteorological Institute).

In Iceland, blue mussels (*Mytilus edulis*) were collected at the reference site Hvalfjörður, and at 6 sites close to or in harbours with supposed different levels of pollution along the south-western and western coast of Iceland. The sites were Reykjavík harbour, Grafarvogur (urban area of Reykjavík), Keflavík harbour, Þorlákshöfn harbour, Ólafsvík harbour, and Ísafjörður (close to a small town and a harbour). The sampling site in Reykjavík is in the old harbour, which is the largest harbour in the country, with intense traffic of small and large vessels. This site was supposed to have the highest marine pollution levels in Iceland. The sampling sites in Keflavík, Þorlákshöfn and Ólafsvík are in small fishing harbours, with supposed intermediate pollution. Grafarvogur is an urban area, situated 5 kilometres east of the cargo harbour in Reykjavík, a site with supposed intermediate to low pollution levels. The sampling site at Ísafjörður is situated 2 km from the small fishing harbour in Ísafjörður town,

with supposed low pollution levels. The reference site Hvalfjörður is situated in a fjord about 60 km from Reykjavík. Mussels from this site have been analysed on a yearly basis within a monitoring program, with results indicating low levels of contaminants. The tidal water range in Iceland is ~3.8m at Reykjavík, Grafarvogur og Hvalfjörður; ~3.7m at Keflavík, Þorlákshöfn and Ólafsvík; and ~2.1m at Ísafjörður. Average sea temperature at these sites in October 2000 was 7-8°C, and salinity 34-35 ‰ (based on data provided by The Marine Research Institute in Iceland).

Mussels sampled

The mussels sampled were adult *Mytilus edulis* ranging from 4-6 cm in size, except for mussels from the Baltic Sea. Baltic Sea mussel populations may contain mussels with a significant proportion of *Mytilus trossulus* genotypes, which are not easily distinguished from *Mytilus edulis*, so here the Baltic Sea mussels are referred to as *Mytilus* spp.. No individuals with pure *M. trossulus* genotypes have been found in the Baltic Sea, but *M. trossulus* alleles are maintained at high frequencies for some allozyme genes (Riginos and Cunningham, 2005). The Baltic Sea mussels have small sizes at maturity, which mostly appears to be a consequence of living in a low salinity environment, and they reach a maximum of 1/3 that of North Sea mussels (e.g. Kautsky 1982). The Baltic Sea mussels sampled here were adult mussels ranging from 2-3cm, which is as large as they get in this area.

There were differences in where from the coastal zone the mussels were sampled. Icelandic mussels were sampled in the intertidal zone at low tide from the shoreline, but in Norway and Sweden where the intertidal range is considerably smaller, the mussels were sampled from just below the tidal range; in the subtidal zone. Swedish mussels were sampled by hand from the shore at 0.5-1m depth, and the Norwegian ones at ~1m depth by diving. This means that the Icelandic mussels sampled were such that are exposed to tidal waters and

the others not. The Swedish and Norwegian mussels are however from shallow waters, and therefore affected by wind and waves, and thus exposed to the surface, although more irregularly, and for shorter periods of time than mussels in the intertidal zone.

Sample preparation

From each sampling site 20 mussels were sampled for DNA-adduct analysis and 25 mussels for analysis of PAHs. After collection, the intestinal canal of the mussels for PAH-analysis were cleaned by keeping them in nets for 24 hours in containers with seawater from the respective sample sites. The temperature was at ambient conditions. The mussels were then frozen for subsequent PAH tissue analysis. Mussels for DNA adduct analysis were transported moist and cold to the laboratory where gills and digestive glands were dissected out and snap frozen in liquid nitrogen. Tissues from five mussels were pooled and ground into a fine homogenous powder in liquid nitrogen by use of a pre-cooled mortar and pestle. An aliquot of the homogenates were used for DNA-extraction. Four pools of tissues were analysed from all sites.

DNA adduct analysis

DNA was extracted from tissue samples and purified essentially as described by Reichert and French (1994). For a detailed description see Skarphedinsdottir et al. (2003). DNA adducts were analysed with the nuclease P₁ version of the ³²P-postlabelling method as described in Reichert and French (1994), with some modifications. Briefly, aliquots of 12.5 µg of purified DNA were hydrolysed with micrococcal nuclease (33 mU/µg DNA) and spleen phosphodiesterase (3.2 mU/µg DNA) at pH 6.0 in incubation for 4 hours at 37 °C. After adjustment of the pH to 5.0, normal nucleotides were hydrolysed to nucleosides by adding 0.5 µg Nuclease P₁/µg DNA and incubating at 37 °C for 30 min. After adjustment of pH to

approximately 7.8, the mixture was evaporated to dryness in a SpeedVac[®] (Savant). Labelling of adducts was performed by adding a mixture containing 5 units of T4 polynucleotide kinase, 3.1 MBq [γ -³²P]-ATP (110 TBq/mmol), 50 mM Tris base, 10 mM MgCl₂, 10 mM dithiothreitol and 5 mM spermidine at pH 7.6. Incubation lasted for 30 minutes at 37°C. Labelled adducts were separated by multidirectional chromatography on laboratory prepared polyethyleneimine cellulose thin layer sheets. Chromatography solvents used for the separation were 1.0 M sodium phosphate at pH 6.0 (D1), 7.25 M urea, 3.86 M lithium formate at pH 3.5 in the opposite direction of D1 (D3), 7.25 M urea, 0.42 M Tris-HCl, 1.36 M lithium chloride at pH 8.0 perpendicular to D3 (D4) and 1.0 M sodium phosphate at pH 6.8 in the same direction as D4 (D5). A PhosphorImager (Molecular Dynamics), was used for detection of the ³²P-postlabelled adducts, and the radioactive spots on the autoradiograms were quantified and converted to digital pixels values with ImageQuant computer software (Reddy and Randerath, 1986).

Three different control samples were run in parallel with all samples: 1) DNA adducted liver tissue from perch liver exposed *in vivo* to benzo[a]pyrene was used as a positive control, processed parallel with the samples to ensure against inter-assay variation, 2) The standard adduct (BaPDE-dG-3'p) was used as a control of labelling efficiency and the chromatographic system, and 3) salmon sperm DNA as a negative control to ensure that no artefacts or false adducts appeared.

Chemical workup and analysis of PAHs in mussel tissue

A total of 32 PAHs were analysed: Naphthalene, 2-methyl-naphthalene, 1-methyl-naphthalene, biphenyl, 2,6-dimethyl-naphthalene, 1,6-dimethyl-naphthalene, 1,5-dimethyl-naphthalene, acenaphthylene, acenaphthene, 2,3,6-trimethyl-naphthalene, 2,3,5-trimethyl-naphthalene, 1,2,4-trimethyl-naphthalene, fluorene, 1,2,3-trimethyl-naphthalene,

dibenzothiophene, phenanthrene, anthracene, 2-methyl-phenanthrene, 1-methyl-phenanthrene, 3,6-dimethylphenanthrene, fluoranthene, 9,10-dimethyl-phenanthrene, pyrene, benz[a]anthracene, chrysene+triphenylene, benzo[b+j,k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3cd]pyrene, dibenz[a,c/a,h]anthracene, benzo[ghi]perylene. From each location a homogenized sample containing soft tissue of 25 individual mussels was prepared. Internal standards were added (deuterated naphthalene, acenaphthene, phenanthrene, chrysene and perylene) to the homogenate and the mixture was saponified. The PAHs were extracted with n-pentane and dried over sodium sulphate. The extraction volume was reduced, solvent exchanged to DCM (dichloromethane), and the extracts were cleaned using gel permeation chromatography (GPC) and solvent was exchanged to cyclohexane. If necessary, the samples were further cleaned by DMF (dimethylformamide) partitioning and silica column elution. The extracts were analysed by GC/MS. The MS detector was operated in selective ion monitoring mode (SIM), and the analyte concentrations in the standard solutions were in the range 5-1000 ng/μl. The GC was equipped with a 30 m column with a stationary phase of 5% phenyl polysiloxane (0.25 mm i.d. and 0.25 μm film thickness), and an inlet operated in the splitless mode. The initial column temperature was 60°C, which after two minutes was raised to 250°C at a rate of 7°C/min and thereafter raised to 310°C at a rate of 15°C/min. The injector temperature was 300°C, the transfer line temperature was 280°C, the MS source temperature was 230°C and the column flow rate was 1.2 ml/min. Internal standards are used for quantification. Standard reference material SRM 2260 from National Institute of Standards and Technology (NIST) has been used for GC-calibration and the relative accuracies have been controlled against SRM 2977. The detection limits depend on sample amount, final extract volumes and instrument conditions. Normally they are 0.5 μg/kg wet weight.

Statistical analysis

DNA adduct levels were compared statistically with levels from each reference site by one-way ANOVA and Newman-Keuls, and Dunnett post hoc test. Relationships between PAHs and DNA adducts were studied with regression analysis, all values were transformed when necessary after checking for normality and homogeneity of the variances. The software used for statistical analysis was Statistica 5.5 (by Statsoft Inc.).

RESULTS**DNA adduct levels**

DNA adduct levels are presented in figure 2, and DNA adduct levels in gills are also shown in table 1. The levels were higher in gills than in digestive gland of the mussels at all sites. The DNA adducts were generally low, or ranging from below the detection limits to 10 nmol adducts/mol normal nucleotides in gills of the subtidally sampled mussels at Fiskaatangen, Norway (harbour and industrial site), and in intertidal mussels at Reykjavík harbour, Iceland. The detection limits are different for each sample, depending on background of the autoradiograms, but calculated average and stdev of detection limits of all the autoradiograms here was 0.43 ± 0.19 nmol DNA adducts/ mol norm nucleotides. DNA adduct levels were significantly elevated in gills of mussels at 6 out of 18 sites compared to reference sites in respective coastal zones. These sites were Fiskaatangen (harbour and industrial site) and Croftholmen (industrial site) in Norway; Reykjavík harbour, Grafarvogur (urbanised) and Þorlákshöfn harbour in Iceland; and Gothenburg (urbanised and harbours), west coast of Sweden. Digestive gland adduct levels were significantly elevated compared to reference sites

at Svartlingsskär (pulp industry), Swedish (east coast) Baltic Sea, Fiskaatangen, Norway, and Reykjavík harbour in Iceland.

The adduct spots on the autoradiograms from gill samples exhibited similar pattern, a diagonal zone on the autoradiograms typical of PAH exposure, see figure 3. Fewer and much fainter adduct spots were observed on autoradiograms from the digestive glands (not shown).

PAH tissue levels

Total PAH tissue levels ($\Sigma 32$ PAHs) are given in table 1. The total PAH tissue levels ranged from 10 – 11670 ng/g dry wt. mussel. The most contaminated mussels were those sampled in the harbours of Reykjavík (11670) and Ólafsvík (10830 ng/g dry wt.), Iceland. Total sum of 4-6 aromatic ring PAHs was highest in Fiskaatangen, Norway (3410 ng/g dry wt.), and Reykjavík harbour, Iceland, which are also the sites that had the highest DNA adduct levels. Total PAH and gill DNA adduct levels were significantly correlated ($r^2 = 0.73$ ($n = 21$, $p = 0.002$)). If individual PAHs are studied, then phenanthrene and pyrene correlated individually best with the DNA adduct levels, with $r^2 = 0.78$ ($p < 0.001$) and 0.71 ($p < 0.001$) respectively. Highest correlation value for a group of compounds was found if the sum of the 4 PAHs 1,6-dimethylnaphthalene, 1,2,4-trimethylnaphthalene, phenanthrene and pyrene was correlated with DNA adducts; $r^2 = 0.78$ ($p < 0.001$). The site Ólafsvík harbour in Iceland is excluded from correlation calculations since it is considered an outlier in this dataset, with very high PAH levels (mainly small PAHs) but relatively low DNA adduct levels.

DISCUSSION

DNA adduct levels

The mussel gill DNA adduct levels reported here from Icelandic sites are in the same range as our previous results on adduct levels in mussels collected from the intertidal zone in Iceland. DNA adduct levels in gills of mussels from Reykjavík harbour were found to be 10.3 nmol/mol norm. nucleotides in August 2000 (Ericson et al, 2002), and little lower or 5.9 nmol/mol norm. nucleotides in October 2001 (Skarphedinsdottir et al., 2005) compared to 10.0 nmol/mol nucleotides in this study (October 2000). Higher adduct levels have been found in mussels in winter at the same site; 18.9 nmol/mol nucleotides in March 2000, and 14.5 in March 2002, which is explained by seasonal variation in native mussels (Ericson et al., 2002; Skarphedinsdottir et al., 2005). Levels in Grafarvogur and Keflavík, Iceland in August 2000 in an earlier study were found to be 4 and 1.5 nmol/mol nucleotides respectively, or similar to our now (October 2000) observed 3.0 and 1.9 nmol/mol nucleotides. Differences in PAH and DNA adduct levels have been demonstrated between intertidal and subtidal mussels, which is important to bear in mind when evaluating and comparing adduct levels in mussels from different areas. The DNA adduct levels were found to be higher in intertidal mussels than in subtidal mussels, with a difference ranging from a factor of 1.3 – 2.9 (Skarphedinsdottir et al., 2005). Since the present study was conducted prior to these findings, depth was not taken into consideration in the sampling of the mussels. The Icelandic mussels here were sampled from the intertidal zone, which can be as large as 3.8m, but the Swedish and Norwegian mussels from just below the considerably narrower intertidal zone in those areas (see methods). The Swedish and Norwegian mussels are however from shallow waters, and therefore affected by wind and waves, and exposed to the surface like the Icelandic mussels, although more irregularly, and for shorter periods of time. But because of these differences, the sites should preferably only be compared with their respective reference site.

Other field studies on DNA adducts in mussels are scarce. Kurelec et al. (1990) found elevated adduct levels in juvenile *M. galloprovincialis* near an oil refinery, but not in adult mussels from polluted sites in the northern Adriatic. Harvey et al. (1997) report 6.1 adducts/ 10^8 nucleotides (tissue not specified) (corresponding to 61 nmol/mol nucleotides) in *M. edulis*, analysed with the nuclease P₁ enhancement method, from sites in Wales, UK. Harvey et al. (1999) studied genotoxic impact of the release of 72,000 tonnes of crude oil from grounding of the Sea Empress oil tanker in Wales, UK, but found no DNA adducts in *M. edulis* 2 and 11 months after the oil spill incident. Soft tissue of whole mussels were analysed for DNA adducts in that study, meaning that if DNA adducts were formed in gills or the digestive gland but not in other tissues, they would appear very diluted. DNA adducts were on the other hand elevated in vertebrates sampled in the area, at least 2 months after the oil spill. Solé et al. (1996) detected DNA adducts ranging from 0.20 to as much as 128 adducts/ 10^8 nucleotides (corresponding to 1280 nmol/mol nucleotides) in digestive gland of *M. edulis* sampled on the Galician coast at industrial and urban sites. Venier et al. (1996) analysed DNA adducts in gills of *M. galloprovincialis* from different sites of the Venice lagoon, and found adduct levels ranging from 0.009 – 0.346 adducts/ 10^8 nucleotides (corresponding to 0.09 – 3.46 nmol/mol nucleotides), which is more in the range of our results. When comparing DNA adduct levels between studies and different laboratories, it is however important to keep some facts in mind. Firstly the fact that DNA adduct levels in mussels are affected by tidal waters, so differences in where from the shoreline the mussels are sampled matter (Skarphedinsdottir et al., 2005). Secondly, the ³²P-postlabelling assay is only a semi-quantitative method. 100% labelling efficiency and adduct resistance to the nuclease P₁ treatment is *assumed*, and depending on the elution system and the hydrophobicity of the adducts, some adducts may be retained at the origin on the TLC sheets, and some washed away and thus not detected. Small

differences in the assay protocol at different laboratories may thus lead to different levels of adducts (Ericson et al., 1998).

Studies on fish from contaminated sites in Nordic coastal areas have generally revealed higher DNA adduct levels than those observed in the mussels in this study, as is to be expected since fish have more active biotransformation system. Several studies on DNA adducts in fish at contaminated sites in the Swedish Baltic Sea have been reported. For example, adduct levels in liver of perch (*Perca fluviatilis*) and pike (*Esox lucius*) in a PAH gradient from an aluminium smelter in the vicinity of the city Sundsvall in the northern part of Swedish Baltic Sea coast ranged from 0.1 – 29 and 3.3 – 13.5 nmol/mol nucleotides respectively (Ericson, et al., 1998). Liver of perch from creosote contaminated site in the river Ångermanälven flowing into the Baltic Sea had up to 6.8 nmol adducts/mol (Ericson, et al., 1999), and liver of perch sampled in the receiving waters of a bleached pulp mill at Norrsundet on the Baltic Sea coast had up to 22 nmol adducts/mol nucleotides (Ericson & Larsson, 2000). The shorthorn sculpin (*Myoxocephalus scorpius*), sampled in 4 harbours of different sizes at the south-west coast of Iceland had up to 32 nmol adducts/mol nucleotides in the liver of fish sampled in the fishing harbour of Sandgerði. Fish sampled in Reykjavík harbour had 23 nmol adducts/mol nucleotides in the liver (Stephensen, et al., 2000), compared with 10.0 in gills of blue mussels from same site in this study. Atlantic cod (*Gadus morhua*) and corkwing wrasse (*Symphodus melops*) caught in the receiving waters of an aluminium works in the Karmsund strait, Norway, revealed up to 27 nmol/mol nucleotides and 239 nmol/mol nucleotides respectively at the most contaminated site (Aas, et al., 2001).

The observed higher DNA adduct levels in gills than in digestive gland are in agreement with our earlier field results (Ericson et al., 2002), and experimental work (Skarphedinsdottir et al. 2003) with blue mussels, where adduct levels have been higher in gills in all cases. Many DNA adduct studies in mussels have focused on the digestive gland, but some

researchers have studied other tissues such as the gills as well. Harvey and Parry (1997) also reported higher adduct levels in gills of *M. edulis* than in digestive gland after experimental exposure to genotoxic compounds. Results on DNA adduct formation in the closely related species *M. galloprovincialis* are however somewhat contradictory; show either no marked differences in adduct levels between these tissues (Canova et al., 1998), or higher BaP-DNA binding in the digestive gland than gills (Akcha et al., 1999). It is possible that the gills are exposed to higher PAH levels through direct absorption into gills from the water phase compared to absorption of PAHs adsorbed to small particles that are taken up via the digestive system. Our experimental work shows however that when exposed to BaP, the mussels accumulate higher BaP levels in the digestive gland, but more DNA adducts are however formed in the gills (Skarphedinsdottir, 2003). The DNA adduct formation is dependent upon the balance between activating (phase I) and detoxifying (phase II) enzymatical processes in the cell, which may vary between tissues. It is thus possible that detoxification of primary metabolites is more effective in digestive gland than in gills of blue mussels or that the digestive gland is more efficient in DNA repairs than gill tissues. Further studies on enzymatical processes in different tissues are needed to elucidate if these are possible explanations for the observed tissue differences in DNA adduct formation in the blue mussel.

PAH tissue levels

The PAH mussel tissue levels in this study are comparable with earlier studies on polluted and clean sites in same or neighbouring areas. Broman et al., (1990) reported $\Sigma 19$ PAHs in mussel tissue sampled on the Swedish coast of the Baltic Sea to be 443 ng/g dry wt. Baumard et al., (1999) analysed $\Sigma 18$ PAH tissue levels in blue mussels sampled near the German coast in the south-west Baltic Sea close to point sources and further out to sea. The levels ranged

between 90 - 3900 ng/g dry wt., or little higher if compared to our $\Sigma 32$ PAH levels of 40 – 1330 ng/g dry wt. in mussels sampled on the Swedish coast of the Baltic Sea. Mussels from mouth of rivers in the Pomeranian Bay and the Gulf of Gdansk in the southern Baltic Sea had PAH levels of 9 – 30 ng/g wet wt. (Potrykus, et al., 2003), compared to 9 – 105 ng/g wet wt. in the Baltic Sea in this study. Granby and Spliid (1995) reported $\Sigma 14$ PAHs in mussels sampled from 25 non point source sites on the Danish coast in the Kattegat in the North Sea, and the belts to be 10 – 111 ng/g wet wt., or similar if compared with our 12 – 91 ng/g wet wt. in mussels although they are sampled from both supposed clean and suspected polluted sites on the Swedish west coast in the Skagerak in the North Sea. Normal background PAH tissue levels in mussel in Norway is reported to be up to 300 ng/g dry wt. and less in more remote areas, but up to as much as 1000 times the background values have been found within outfalls of industries, such as smelter discharges. PAH tissue levels in blue mussels from the contaminated Saudafjord have for example been found to range from 8300 - 1729000 ng/g dry wt. (Næs et al., 1995), compared to 720 – 5120 ng/g dry wt. in Norwegian mussels in this study. PAH tissue levels in blue mussels transplanted to the intertidal zone in Reykjavík harbour 2001 ranged from 9949 – 114294 ng/g lipid weight (corresponding to 845 – 9715 ng/g dry wt.) with maximum levels in winter, and minimum levels in spring and summer (Skarphedinsdottir, et al. 2005).

Mussel PAH tissue levels from other locations for comparison are for example 8 – 341 wet wt. in *M. edulis* sampled at both diffuse and point sources at coastal locations in Scotland, and 15 – 7200 ng/g wet wt. from Shetland and Orkney (Webster et al., 2003) (compared with our 3 – 1559 ng/g wet wt. at all locations). Solé (2000) reported PAH tissue levels in mussels from pollution gradients from harbours in the Mediterranean coast, Venice lagoon (*M. galloprovincialis*), Galician coast, and the North Sea (*M. edulis*) to be 19-2675, 17-139, 22-270, and 4-16 ng /g wet wt. respectively. Baumard et al., (1998) studied levels of 14 PAHs in

M galloprovincialis from 10 remote and polluted sites of the Mediterranean coasts of Spain and France, and found them to range from from 25-337 ng/g dry wt. Porte et al., (2001) reported also PAH levels in *M. galloprovincialis* from a pollution gradient on the Galician coast (NW Spain), which were found to be 22 - 562 ng/g wet wt. Mussels from western Greenland contained 497 ng/g dry wt. (Pecseli et al. 2002). In general, our PAH levels seem correspond to the less and moderately polluted areas of Europe.

Comparison of mussel pollutant tissue levels between studies should however be practiced with caution, since many factors affect the pollution tissue levels, such as intertidal impact, seasonal variation in filtering rates, reproduction and lipid levels. Filtering rates are higher during spring and autumn algae bloom, which induces greater exposure to contaminants, and spawning in spring/ summer releases lipids as well as hydrophobic contaminants (Gosling, 1992).

Of individual PAHs, phenanthrene and pyrene correlated individually best with the DNA adduct levels, with $r^2 = 0.78$ ($p < 0.001$) and 0.71 ($p < 0.001$) respectively, and highest correlation value for a group of compounds was found if the sum of the 4 PAHs 1,6-dimethylnaphthalene, 1,2,4-trimethylnaphthalene, phenanthrene and pyrene was correlated with DNA adducts; $r^2 = 0.78$ ($p < 0.001$). Most of the DNA adduct and PAH tissue levels in this study are low, and higher values are actually needed for more reliable correlation calculations and interpretation of the relationship between DNA adducts and tissue levels. The ^{32}P -postlabelling method used here for analysing the DNA adducts, with the nuclease P_1 version and standard elution system, is traditionally considered to detect mainly large hydrophobic aromatic (4-6 ring) DNA adducts. Therefore, the smaller PAHs (like naphthalene) are probably not contributing directly to the DNA adduct levels. They could however affect the levels indirectly, e.g. by inducing the bioactivating system and in that way possibly lead to higher levels of larger DNA-reactive intermediates.

The relationship between PAH tissue levels and its genotoxic effects as seen in DNA adducts is also complicated by several factors. PAHs have to be metabolised to form DNA adducts in organisms, so the parent compound is no longer measurable when its reactive metabolite exerts its genotoxic effects by binding to DNA and forming adducts. Organisms are also known to accumulate and biotransform compounds selectively. For example, benzo[e]pyrene has been observed to be preferentially accumulated relative to benzo[a]pyrene by *M. edulis* (Baumard 1999) and *M. galloprovincialis* (Baumard et al. 1998). This has been related to selective biotransformation of B[a]P, while B[e]P is preferentially accumulated in the lipids. Benzo[a]pyrene has also been shown to be more genotoxic than benzo[e]pyrene, so the higher B[e]P values could be attributed to a preferential biotransformation of B[a]P to more reactive and genotoxic metabolites.

Origin of the PAHs

Mussels sampled in or close to harbours had relatively higher levels of lower molecular weight PAHs, while mussels sampled close to industrial areas had higher levels of larger PAHs. Ratios between individual PAH compounds have also been used as a way of indicating the processes from which PAHs originate. PAHs are generated by three main processes: Combustion at high temperature of organic matter, release of petroleum, and degradation of organic matter (Neff, 1979). Phenanthrene/Anthracene (Phe/Ant) and Fluorene/Pyrene (Fl/Pyr) ratios are commonly used as a means of determining the dominant origins of PAHs (Colombo et al., 1989; Budzinski et al., 1997). Pyrolysis of organic matter at high temperatures generates PAHs characterised by low Phe/Ant ratio (<10), while the slow maturation of petroleum at lower temperatures leads to larger values of the Phe/Ant ratio (> 25) (Baumard et al., 1999). Fluoranthene and Pyrene are often associated and considered typical pyrogenic products generated from high temperature condensation of low molecular

weight compounds (Soclo et al., 2000). During the combustion process pyrene is more stable than fluoranthene, so pyrolytic products are usually characterised by a predominance of fluoranthene over pyrene at ratios >1 (Baumard et al., 1999). So $Ph/An > 10$ and $Fl/Pyr < 1$ indicates petrogenic origin of the PAHs, while the opposite indicates pyrogenic origin.

The Phe/Ant and Fl/Pyr ratio values in this study (see table 1) indicated that the PAHs were in most cases of pyrolytic origin, but with input of petrogenic hydrocarbons close to harbours and the oil refinery (indicated by Fl/Pyr ratios) as seen in the Icelandic mussels sampled in or close to harbours, in mussels sampled in Gothenburg, Swedish west coast (river mouth; urbanised and harbours), and in Brofjorden, Swedish west coast near an oil refinery. According to these ratio values, mussels from other sites in the study have mainly been exposed to PAHs with pyrolytic origin, or from industries, traffic and fuel combustion.

CONCLUSIONS

The objective of this field study was to characterise PAH tissue levels and their genotoxic effects as seen in DNA adducts in blue mussels (*Mytilus* spp.) in Nordic coastal areas. Few papers have reported on DNA adduct and PAH levels in blue mussels in the field, especially from these areas, although mussels are important components of the coastal ecosystems and are in many ways ideal bioindicator organisms. In this study, mussels were sampled from 22 sites in Iceland, Norway and Sweden that were suspected to have various PAH origins, mixtures and levels, such as harbours of different sizes, industrialised and urban sites, as well as pristine sites for reference. The results revealed that DNA adducts were higher in gills than digestive gland of the mussels at all sites studied. PAH tissue levels and DNA adducts were generally low, indicating low PAH exposure to mussels in the areas studied. Total PAH tissue

levels correlated well with the DNA adduct levels ($r^2= 0.73$). The PAHs that correlated individually best with DNA adduct levels were phenanthrene ($r^2= 0.78$) and pyrene ($r^2= 0.71$). According to PAH ratio values, the PAH found in the mussel tissues were mainly of pyrolytic origin, but with petrogenic input close to most harbours and the one site studied near an oil refinery.

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Table 1. Description of the sampling sites, pollution sources at the sites, size of sampled mussels, average DNA adduct levels in gills of the mussels, PAH whole mussel tissue levels, and PAH ratio values of phenanthrene/ anthracene and fluorene/pyrene

Site no.	Coastal area	Samplingstation	Expected pollution source	Mussel size mm average \pm stdev	DNA adducts (nmol/mol norm. nucl.)	Sum PAH ng/g dry wt (ng/g wet wt)	Ph/An	Fl/Py
1	Iceland	Hvalfjörður (reference)	Remote, no site specific source	48.8 \pm 3.0	0.53	240 (35)	5.8	1.4
2	Iceland	Ísafjörður	Small fishing harbour	45.1 \pm 5.2	1.50	230 (20)	6.6	0.7
3	Iceland	Ólafsvík	Small fishing harbour	53.5 \pm 5.0	2.10	10830 (1559)	3.9	0.2
4	Iceland	Grafarvogur	Urbanised, 5 km from cargo harbour	44.1 \pm 3.4	3.00	560 (73)	4.2	0.5
5	Iceland	Reykjavík harbour	Harbour, urbanised	43.4 \pm 4.0	10.00	11670 (1891)	5.6	0.7
6	Iceland	Keflavík	Small fishing harbour	49.5 \pm 4.4	1.90	n.a.	-	-
7	Iceland	Þorlákshöfn	Small fishing harbour	46.7 \pm 3.9	4.10	3520 (395)	1.3	3.0
8	Norway	Valløy (reference)	Remote, no site specific source	59.4 \pm 4.9	1.20	723 (103)	10.2	1.1
9	Norway	Fiskaatangen	Harbour, anode plant for Mn smelters	55.9 \pm 3.9	10.10	5120 (574)	6.1	1.1
10	Norway	Croftolmen	Inner fjord-harbour, Mn smelter	53.7 \pm 3.6	3.10	1315 (119)	4.8	1.1
11	Norway	Sauda	Inner fjord/harbour, Mn smelter	40.7 \pm 4.1	0.91	1240 (177)	4.1	1.7
12	Sweden, West coast	Fjällbacka (reference)	Remote, no site specific source	49.1 \pm 5.2	0.58	240 (41)	7.5	1.4
13	Sweden, West coast	Brofjorden	Oil refinery	48.8 \pm 4.7	1.20	370 (62)	2.8	0.9
14	Sweden, West coast	Stenungsund a (Jordh.bukten)	Petrochemical industry	53.3 \pm 5.5	2.40	125 (12)	4.0	1.5
15	Sweden, West coast	Stenungsund b (Almön)	Petrochemical industry	51.4 \pm 4.7	1.40	120 (19)	4.9	1.2
16	Sweden, West coast	Gothenburg a (Nya Varvet)	Urbanised river mouth, harbours	49.7 \pm 4.1	2.80	925 (77)	4.5	0.9
17	Sweden, West coast	Gothenburg b (Sandvik)	Urbanised, small harbour	52.3 \pm 3.9	2.30	660 (91)	5.1	1.1
18	Sweden, Baltic Sea	Askö (reference)	Remote, no site specific source	24.1 \pm 2.2	0.90	40 (3)		
19	Sweden, Baltic Sea	Oxelösund	Steel plant	22.6 \pm 1.8	2.60	1330 (105)	5.8	1.9
20	Sweden, Baltic Sea	Kväddöfjärden	Remote, no site specific source	26.2 \pm 1.6	3.80	100 (9)	1.2	1.6
21	Sweden, Baltic Sea	Svartören	Pulp industry	27.5 \pm 2.1	1.40	320 (24)	3.6	1.5
22	Sweden, Baltic Sea	Svartlingskär	Pulp industry	24.2 \pm 2.4	3.30	220 (16)	3.2	1.7

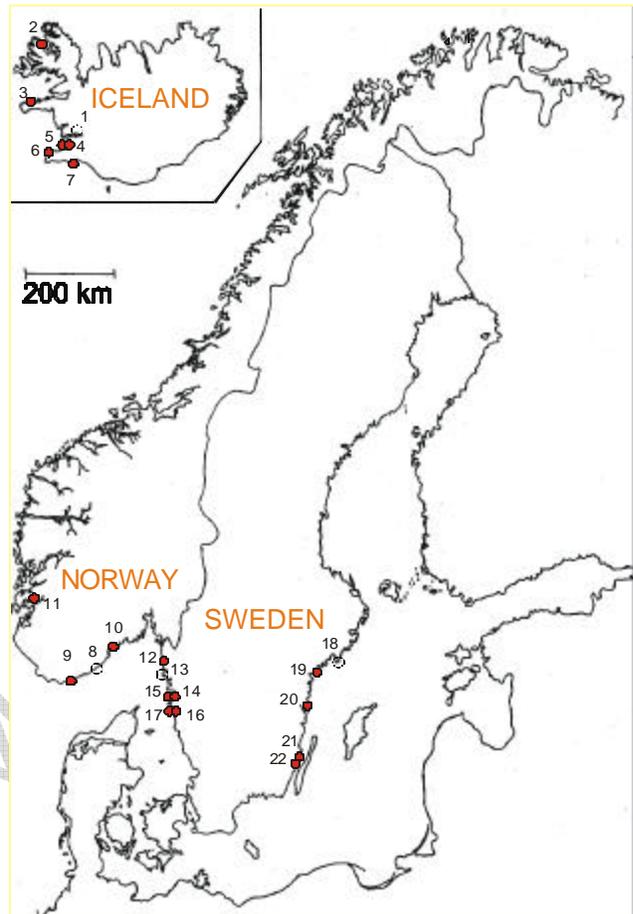


Figure 1. Sampling sites.

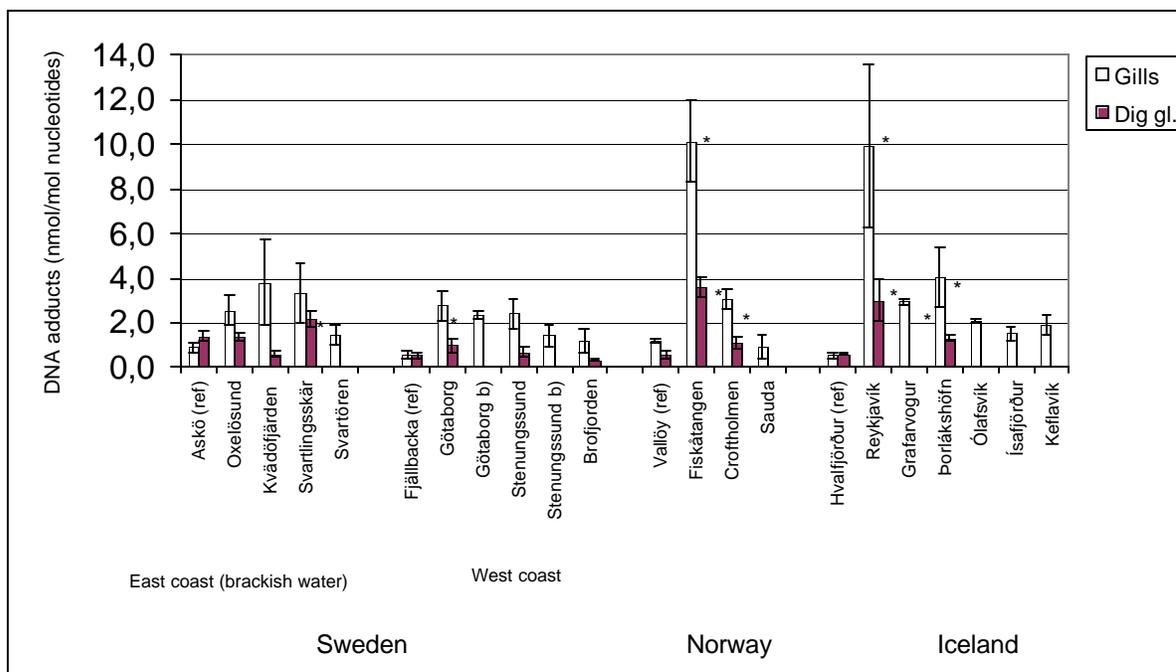


Figure 2. DNA adduct levels in gills and digestive gland of *Mytilus* spp. in Sweden, Norway and Iceland (mean \pm SE, $n = 3 - 4$ pools of 5 mussels each). * = $P < 0.05$, significantly different from respective reference site (which is the first sites/bars in each region).

ACCEPTED MANUSCRIPT

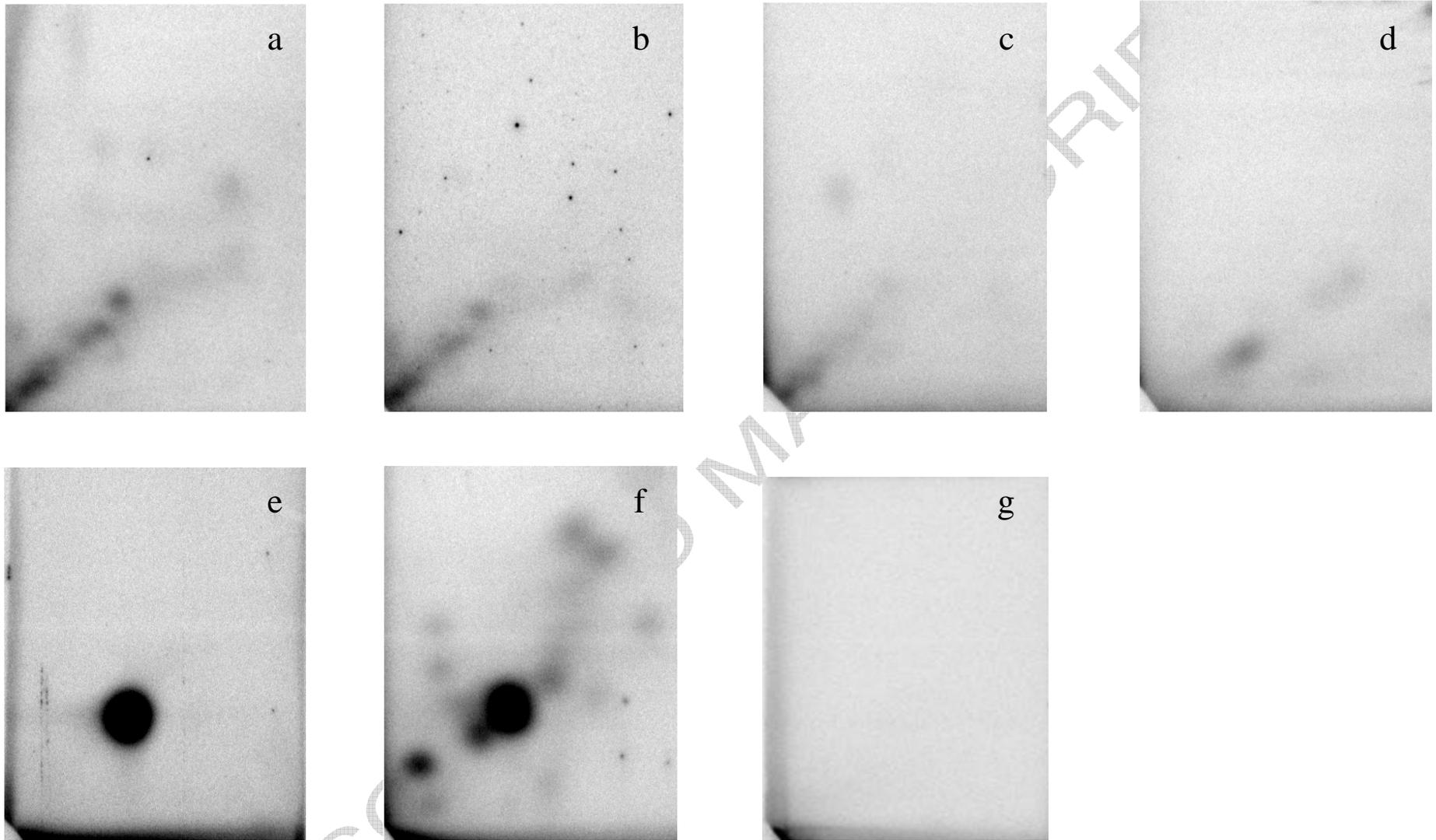


Figure 3. Representative autoradiograms of ^{32}P -postlabelled DNA adducts in gills of *Mytilus edulis* from Reykjavík harbour, Iceland (a); Fiskaatangen, Norway (b); Göteborg, west coast of Sweden (c); and *Mytilus* spp. from Kvädöfjärden, east coast of Sweden, The Baltic Sea (d); the standard BaP-dG adduct (e); the positive control sample from perch exposed to benzo[a]pyrene (f); and the negative control of salmon sperm (g).