BIOMARKERS, 1998, VOL. 3, NO. 1, 49–62

Responses of the cytochrome P450-dependent monooxygenase and other protective enzyme systems in digestive gland of transplanted common mussel (*Mytilus edulis* L.) to organic contaminants in the Skagerrak and Kattegat (North Sea)

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Received 25 June 1997, revised form accepted 28 September 1997

In order to determine the biological impact of contaminants in the Skagerrak and Kattegat, mussels (*Mytilus edulis* L.) (4·5–6 cm in length) from a clean area (Faroe Islands) were transplanted for 6–8 weeks in 1993 and 1994 to sites in the Faroe Islands (reference control), to the Skagerrak deep-water region between Norway and Sweden, and to suspected contaminant-influx sites near the Hvaler Archipelago (Norway) and Göteborg (Sweden). Similar results were obtained in both years. Whole body total polynuclear aromatic hydrocarbons (PAHs) were 57–206 % higher in *M. edulis* from the Skagerrak, Norway and Sweden sites (up to 62 ng g^{-1} dry wt) compared with the Faroe Islands reference control, whereas no differences were seen in organochlorines (PCBs, chlordanes, DDTs, hexachlorocyclohexanes, hexachlorobenzene). Digestive gland microsomal benzo[*a*]pyrene hydroxylase (BPH) activity (formation of phenols) was elevated at all the contaminated sites compared with the Faroe Islands reference control ($p < 0{\cdot}05$). BPH turnover (BPH activity pmol $^{-1}$ P450) was elevated 132–288 % compared with the the Faroe Islands ($p < 0{\cdot}05$) and showed limited correlation with total PAHs ($r^2 = 0{\cdot}58$). Overall, the results are indicative of impact by PAHs and induction of the cytochrome P450 monooxygenase system. In contrast to previous studies on *M. edulis* exposed to higher tissue levels of PAHs or PCBs, no elevation of cytochrome P4501A-immunopositive protein (CYP1A) was detected using antibodies to fish hepatic CYP1A. Little or no differences between any sites were seen in digestive gland glutathione S-transferase (EC 2.5.1.18), superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6) activities.

Keywords: molecular biomarkers, *Mytilus edulis*, cytochrome P450 monooxygenase system, benzo[*a*]pyrene hydroxylase, Skagerrak, Kattegat, North Sea.

Abbreviations: BaP, benzo[a]pyrene; BPH, benzo[*a*]pyrene hydroxylase; CDNB, 1-chloro-2,4-dinitrobenzene; CYP1A, cytochrome P4501A; GSH, reduced glutathione; GST, glutathione S-transferase; HCB, hexachlorobenzene; HCH, hexachlorocychlohexane; HPLC, high performance liquid chromatography; IgG, immunoglobulin G; MFO, mixed-function oxygenase; NADPH, β-nicotinamide adenine dinucleotide phosphate reduced form; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorobiphenyl; ROS, reactive oxygen species; SOD, superoxide dismutase.

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A preliminary report of part of the data was published in Förlin *et al.* 1996, *Marine Environmental Research*, **42**, 209–212.

1354-750X/98 $12.00

Introduction

The Skagerrak and Kattegat are areas of deep sea between Norway, Sweden and Denmark (figure 1). Studies on fish in the mouths of the rivers Göta älv (outside Göteborg, Sweden) (Förlin and Celander 1993) and Glomma (Hvaler Archipelago, Norway) (Goksøyr *et al.* 1991) have identified these estuaries as important entry routes of pollution into Skagerrak. Since the Skagerrak is a suspected sink for anthropogenic contaminants (Förlin *et al.* 1996a), and elevated levels of polycyclic aromatic hydrocarbons (PAHs) and various organochlorines have been observed in sediments from Skagerrak and Kattegat compared with the more pristine Faroe Islands (Magnusson *et al.* 1996, Cato and Karlsoon 1997), a series of ecotoxicological studies was carried out in 1993 and 1994 by the Swedish Environment Protection Agency to determine the biological impact of contaminants in Skagerrak and Kattegat (under the project 'Large-scale Environmental Effects and Ecological Processes in Skagerrak-Kattegat'). Included in this study was the use of transplanted common mussel, *Mytilus edulis* (L.), as an indicator of sediment contaminant bioavailability and environmental health (Förlin *et al.* 1996a).

The integrated measurement of chemical contaminant levels and biomarker responses has been extensively used in pollution monitoring and impact assessment in aquatic environments (McCarthy and Shugart 1990, Huggett *et al.* 1992, Livingstone 1993). Early warning molecular and cellular biomarkers serve to prevent deleterious effects occurring at higher levels of biological organization, such as the population level, by allowing protective measures to be taken (Förlin *et al.* 1996b). Both indigenous and transplanted mussels have been widely used in pollution monitoring because of their sessile nature and ready uptake and bioaccumulation of organic contaminants (Walker and Livingstone 1992, Widdows and Donkin 1992). In the current study, adult *M. edulis* from the Faroe Islands were transplanted in cages for 6–8 weeks to sites near to the point of collection in the Faroe Islands (reference control site), in the deep waters of the Skagerrak, and near to the suspected influx of contaminants from the Hvaler Archipelago, Norway and Göteborg, Sweden (see Materials and Methods for site details). Chemical contaminants measured in the tissues of *M. edulis* comprised PAHs, polychlorobiphenyls (PCBs), DDTs and other organochlorines. Biomarker responses to such contaminants were assessed principally in terms of the cytochrome P450-dependent monooxygenase, or mixed-function oxygenase (MFO) system of the digestive gland, which is the major organ of organic xenobiotic metabolism, antioxidant and biotransformation enzyme activities in *M. edulis* (Livingstone 1996). Although induction of hepatic cytochrome P4501A ('CYP1A') is routinely used in vertebrates, such as fish, as a specific biomarker of exposure to organic contaminants such as PAHs, PCBs and related compounds (Bucheli and Fent 1995, Goksøyr 1995), much less is known of the application of MFO-related measurements in molluscs and other marine invertebrates (Livingstone 1996, Livingstone and Goldfarb 1997). However, recent immunorecognition and molecular biological studies have indicated the existence of an inducible CYP1A-immunopositive protein (N.B.: no sequence data yet exist to categorically identify the enzyme as CYP1A), possibly involved in the metabolism of the model substrate benzo[*a*]pyrene (BaP), in digestive gland of *Mytilus* sp. (Michel *et al.* 1993, Wootton *et al.* 1996, Canova *et al.* 1997, Livingstone *et al.* 1997). Additionally, measurements of '418-peak' (putative

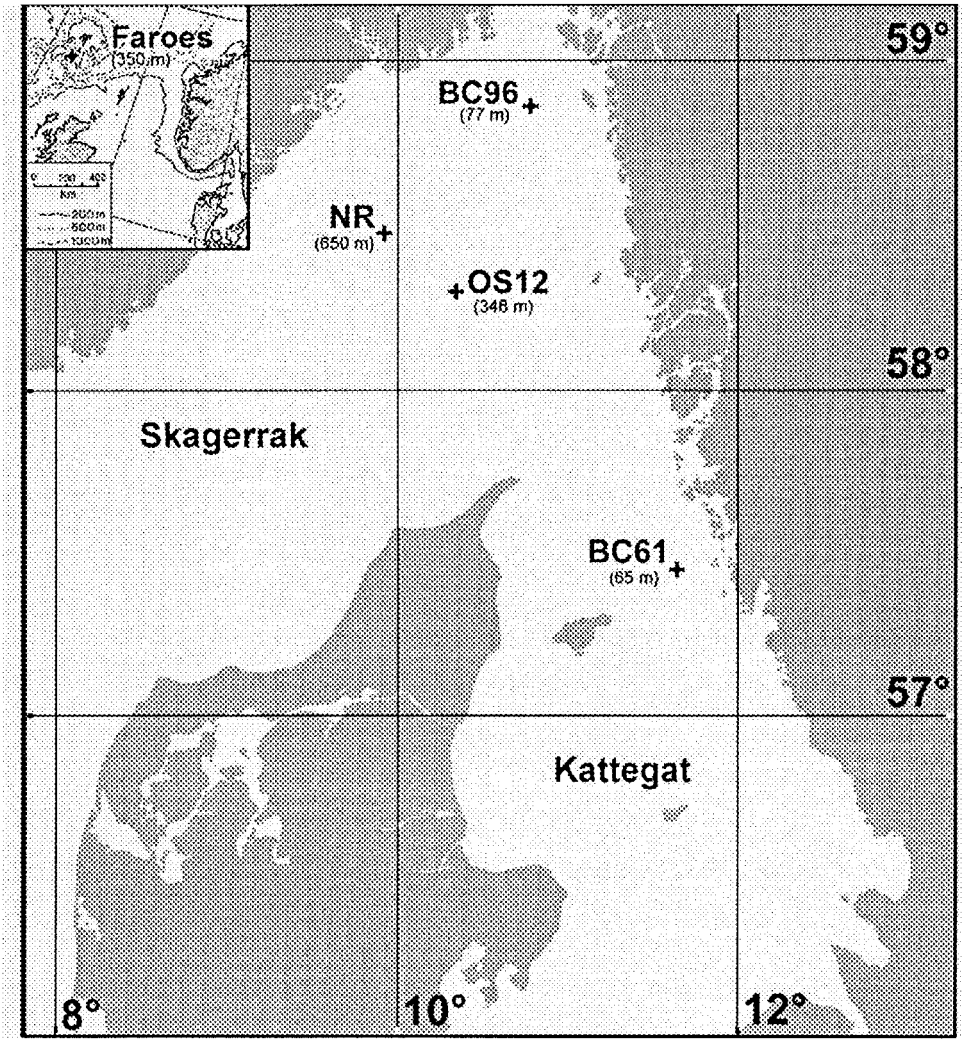

Figure 1. Map of Skagerrak and Kattegat showing location of *M. edulis* transplanted in cages from the Faroe Islands to deep regions of the Norwegian Trench (sites NR and OS12; 700 m and 350 m depth), outside Oslo Fjord (site BC96; 80 m depth) and 30 km from Göteborg (site BC61; 70 m depth); the reference transplant site was near to the original collection site in the Faroe Islands (60 m depth).

denatured cytochrome P450), total cytochrome P450, BaP hydroxylase activity and CYP1A-immunopositive protein have been successively used in a number of field studies of *Mytilus* sp. with exposure to industrial, urban or oil pollution (Livingstone 1988, Narbonne *et al.* 1991, Michel *et al.* 1994, Livingstone *et al.* 1995, Solé *et al.* 1995a, 1996). Also, studied in the transplanted *M. edulis* were the activities of the phase II conjugase glutathione S-transferase (GST; EC 2.5.1.18) and the antioxidant enzymes superoxide dismutase (SOD; EC 1.15.1.1) and catalase (EC 1.11.1.6), which have also been used as potential biomarkers in previous field studies (Porte *et al.* 1991, Sheehan *et al.* 1991, Livingstone *et al.* 1995, Fitzpatrick *et al.* 1997). The main aims of the study were to obtain information on the presence and biological effects of organic contaminants in the

Skagerrak and Kattegat, and to further develop the use of the MFO system and other enzyme measurements as specific biomarkers of organic pollution in bivalve molluscs.

Materials and methods

Animals and tissue collection

Adult *M. edulis* (4·5–6 cm shell length) of mixed-sex were collected from a relatively clean site at the Faroe Islands and transplanted in 50 cm × 30 cm square cages placed on the seabed. Each cage containing 70 *M. edulis* was secured to the seabed by an anchor, and identified via a rope to the surface to which was attached a buoy equipped with a radar reflector and a flag. The studies were carried out between July and September in both 1993 and 1994. The sites are shown in figure 1 and comprised one near the original collection site in the Faroe Islands as the reference site (site F; 60 m depth), and four in the Skagerrak—two in the deep regions of the Norwegian Trench (sites NR and OS12; respectively 650 and 346 m depths), and one each outside the Oslo Fjord (site BC96; 77 m depth) and 30 km from Göteborg (site BC61; 65 m depth). Sites F and NR were sampled in both 1993 and 1994, BC96 and BC61 in 1993, and OS12 in 1994. Three cages were placed at each site, but not all were recovered (see Results), and the *M. edulis* were exposed at the sites for 6–8 weeks before recollection. Following recovery of the cages, the *M. edulis* were immediately dissected on board and whole tissues were stored at –75 °C for contaminant analysis, and digestive glands were damp-dried, frozen in liquid nitrogen and then similarly stored at –75 °C prior to biochemical and immunochemical analysis.

Chemicals

Biochemicals, including β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH), reduced glutathione (GSH), BaP, horse heart cytochrome c, hypoxanthine, hydrogen peroxide (H_2O_2), and 1-chloro-2,4-dinitrobenzene (CDNB), goat anti-rabbit IgG (whole molecule) alkaline phosphatase conjugate and xanthine oxidase (EC 1.2.3.2) were obtained from Sigma Chemical Co., UK. All other chemicals, including organic solvents, were of AnalaR grade, or equivalent, and were obtained from Merck, UK or equivalent. Nitrocellulose was from Amersham, UK; PD-10 Sephadex G-25 columns from Pharmacia-LKB, UK; and rabbit polyclonal antibody to hepatic CYP1A of perch (*Perca fluviatilis*) was a kind gift from Professor L. Förlin, University of Göteborg, Sweden.

Chemical analyses

The detailed analyses of individual PAHs and organochlorines of the 1993 trip have been published elsewhere (Förlin *et al.* 1996a). Only the sum of the individual components of the major contaminant groups are presented here. Organic contaminants were extracted from the whole tissues of 15 pooled animals per site and analysed as described in Brorström-Lundén *et al.* (1994). Tissues were homogenized using an Ultra-Turrax cutting blade homogenizer and contaminants sequentially extracted twice with 20 ml of acetone each time and then with 20 ml of dichloromethane using ultrasonication. The combined extracts were shaken with water (2 : 1 v/v) containing 2 % Na_2SO_4. Organochlorines, including PCBs, hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB), were analysed by gas chromatography and electron capture detection, and PAHs by HPLC and fluorescence detection, using appropriate internal standards.

Biochemical analyses

The pooled digestive glands of four to six mussels were used for each replicate sample, and five replicate samples were prepared per cage. Subcellular samples were prepared at 4°C by differential centrifugation as described in Livingstone (1988). Frozen tissues were homogenized using an electrically-driven Potter-Elvehjem homogenizer in 1 : 4 tissue weight : buffer volume of 10 mM Tris–HCl pH 7·6, 0·15 M KCl, 0·5 M sucrose. Cytosolic and microsomal fractions were obtained in respectively homogenization buffer and 10 mM Tris–HCl pH 7·6, 20 % (w/v) glycerol at protein concentrations of approximately 10 mg ml^{-1} by differential centrifugation at 500*g* × 15 min, 10 000*g* × 45 min and 100 000*g* × 90 min. Biochemical measurements were carried out either immediately (cytosolic fractions), or after overnight storage in liquid nitrogen (microsomes).

All assays were carried out in duplicate. Enzyme activities were measured at 25 °C, and were linear with time and a 5- to 10-fold range of sample concentration. MFO components and activities were measured on microsomes as follows. Total cytochrome P450 and '418-peak' (putative denatured cytochrome P450) contents were assayed by the carbon-monoxide difference spectrum of sodium dithionite reduced sample as described in Livingstone (1988) using a extinction coefficient of 91 $mM^{-1}cm^{-1}$ for cytochrome P450. CYP1A-immunopositive protein was measured by Western blotting

according to Towbin *et al.* (1979) as described in Porte *et al.* (1995), using polyclonal antibody to hepatic CYP1A of *P. fluviatilis* and quantified by image analysis; positive controls for the Western blotting were partially purified P450 from digestive gland of *M. edulis* (Porte *et al.* 1995) and hepatic microsomes from β-naphthoflavone-induced turbot (*Scophthalmus maximus*) (Peters and Livingstone 1995). BaP hydroxylase activity was assayed in the presence of NADPH by the fluorometric assay of Dehnen *et al.* (1973) (measures predominantly phenols—excitation: 467 nm; emission: 525 nm) as described in Livingstone (1987). Assay conditions in a final volume of 1 ml were 50 mM triethanolamine–HCl pH 7·6, 10 mM $MgCl_2$, 60 μM BaP (in 40 μl dimethylformamide), 0·2 mM NADPH and about 1 mg microsomal protein. Reactions were started by the addition of BaP and terminated after 10 min by 1 ml cold acetone. GST, catalase and SOD activities were assayed spectrophotometrically in the cytosolic fraction after passage down a Sephadex G-25 column to remove the small molecular weight fraction (< 10 kDa) which may interfere with the SOD assay (Livingstone *et al.* 1992). GST activity was assayed using CDNB as substrate and measuring the formation of the conjugate product at 340 nm as described in Fitzpatrick and Sheehan (1993). Catalase and SOD activities were assayed as described in Livingstone *et al.* (1992), the former by the decrease in absorbance at 240 nm due to H_2O_2 consumption (ext. coeff. 40 $M^{-1}cm^1$), and the latter by inhibition of the reduction of cytochrome c by hypoxanthine/xanthine oxidase-generated superoxide anion radical (O_2^-) (one unit of SOD activity is defined as the amount of sample causing 50 % inhibition under the standard conditions of the assay) (McCord and Fridovich 1969). The standard assay conditions were *GST*: 0·2 M K_2HPO_4/KH_2PO_4 pH 7·9, 0·2 mM CDNB, 0·2 mM GSH; *catalase*: 50 mM K_2HPO_4/KH_2PO_4 pH 7·0, 50 mM H_2O_2; *SOD*: 43 mM K_2HPO_4/KH_2PO_4 pH 7·7, 0·1 mM EDTA, 50 mM hypoxanthine, 5·7 mU xanthine oxidase, 10 mM cytochrome c. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical treatment

The results are presented as mean ± range or ± SEM (see text for numbers of samples). Differences between groups of values were tested by multivariance one way ANOVA analysis, $p < 0{\cdot}05$ was accepted as statistically significant.

Results

The levels of major groups of organochlorine and other organic contaminants present in whole tissues of *M. edulis* following transplantation for 6–8 weeks from the Faroe Islands to various sites in the Skagerrak, Kattegat and Faroe Islands in 1993 and 1994 are shown in table 1. Only single analyses of pooled material were available for 1993, whereas multiple analyses of material from separate cages at each site were carried out in 1994. Differences in contaminant levels between sites were clearly evident only for total PAHs (sum of eleven 3–5 ring compounds), which were indicated to be 139–206 % higher in the Norwegian Trench (site NR) and near to Oslo Fjord (BC96) and Göteborg (BC61) compared with the Faroe Islands in 1993, and were 57 % higher in NR than the Faroe Islands in 1994 because of an elevation on the pollutant levels at the control site. There was some indication of elevated organochlorines (HCHs, HCB, DDT and metabolites) in the Skagerrak compared with the Faroe Islands in 1993, but this was not confirmed in 1994. No differences or trends were evident in total PCBs (sum of seven major congeners) between sites. Overall, the results indicate the Faroe Islands as a suitable reference site with respect to the bioaccumulation of PAHs.

The levels of MFO components and activities in digestive gland microsomes of transplanted *M. edulis* for 1993 and 1994 are presented in tables 2 and 3 respectively. Results are given both for individual cages and the total pooled data for all cages at each site. Total cytochrome P450 content was 42 % lower at site NR than the Faroe Islands site in 1993 (table 2); lower cytochrome P450 content was also indicated in other Skagerrak and Kattegat sites relative to the Faroe Islands site, both in 1993 (sites BC96 and BC61) and 1994 (sites NR and OS12), but the differences were not statistically significant. No differences were seen between sites

Table 1. Levels of organic contaminants (ng g^{-1} dry wt) in whole body *M. edulis* transplanted for 6–8 weeks from Faroe Islands to sites in Faroe Islands, Skagerrak and Kattegat during 1993 and 1994[1].

Year and site	PAHs[2]	PCBs[3]	DDTs[4]	Chlordanes[5]	HCHs/HCB[6]
1993					
Faroe	17·7	7·6	3·20	1·51	0·95
NR	44·0	6·7	3·45	1·96	1·08
BC96	42·7	8·1	4·47	1·50	1·23
BC61	54·6	10·5	5·30	1·72	1·75
1994					
Faroe (3)	39·8 ± 2·5	9·6 ± 0·3	3·21 ± 0·21	1·48 ± 0·03	0·83 ± 0·04
NR (3)	62·3 ± 3·7*	9·4 ± 1·1	3·39 ± 0·31	1·63 ± 0·21	1·07 ± 0·11
OS12 (2)	54·4 ± 10	8·6 ± 0·8	2·95 ± 0·16	1·46 ± 0·28	0·98 ± 0·14

[1]Values are for single determinations (1993) or means ± range or SEM (number of samples given in parenthesis after site name), $*p < 0{\cdot}05$ compared with Faroe; [2]total polycyclic aromatic hydrocarbons (PAHs; sum of phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*a*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[*cd*]pyrene); [3]total polychlorobiphenyls (PCBs; sum of congeners CBs-28, 52, 101, 118, 138, 153 and 180); [4]sum of *pp′*-DDT, *pp′*-DDE, *pp′*-DDD; [5]sum of α-chlordane, γ-chlordane and *trans*-nonachlordane; [6]sum of α-, β- and γ-hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB).

in levels of '418-peak' and CYP1A-immunopositive protein in either 1993 or 1994. In contrast, BPH activity was 116–152 % higher in individual and/or pooled cages at sites NR, BC96 and BC61 compared with the Faroe Islands site in 1993 (table 2), and were indicated to be higher at sites NR and OS12 compared with the Faroe Islands in 1994. The differences in BPH activity in Skagerrak and Kattegat compared with the Faroe Islands were most marked when expressed in terms of substrate turnover, i.e. BPH activity per amount of cytochrome P450, which showed elevated pooled site values over the Faroe Islands site of 288 % and 132 % (NR, 1993 and 1994), 202 % (BC96), 141 % (BC61) and 156 % (OS12) (tables 2 and 3). Elevations were also seen comparing individual cages at the Skagerrak and Kattegat sites with those at the Faroe Islands (see tables 2 and 3). Pooling the data for all sites and both years, a positive correlation was seen between BPH turnover and total PAHs (figure 2; $r^2 = 0{\cdot}58$). Comparing the results for the two years (compare tables 2 and 3), higher levels of total cytochrome P450 content, '418-peak' and BPH activity were seen, or indicated, in 1994 compared with 1993, whereas BPH turnover was very similar for both time points. CYP1A-immunopositive protein levels were also indicated to be higher in 1994 than 1993, but the comparison is only semi-quantitative because the analyses for the 2 years were carried out on separate gels in separate experiments. GST and antioxidant activities were measured in 1993 only and the results are presented in table 4. GST activity was the same at all sites, with the exception of a lower activity outside Göteborg (at site BC61). Catalase activity was the same at all sites, whereas SOD activity was lower (at site BC61), or indicated to be lower (at sites NR and BC96), in the Skagerrak and Kattegak compared with the Faroe Islands site.

Discussion

The Skagerrak is thought to be a sink both for air-borne and locally derived contaminants and those brought by the out-flowing Baltic and Jutland currents

Table 2. Responses of microsomal MFO system components and activities in digestive gland of *M. edulis* transplanted for 6–8 weeks from Faroe Islands to sites in Faroe Islands, Skagerrak and Kattegat during 1993.

Site and cages	Total P450[1]	'418-peak'[2]	'CYP1A'[3]	BPH[4]	BPH turnover[5]
Faroe	30·4 ± 6·0	8·89 ± 1·00	5·76 ± 0·30	11·6 ± 3·6	0·41 ± 0·09
NR cage 1	15·6 ± 1·0*	8·02 ± 0·64	5·13 ± 1·01	27·7 ± 6·4*	1·91 ± 0·58*
NR cage 2	21·3 ± 4·1	8·15 ± 0·66	4·56 ± 0·56	23·1 ± 4·9	1·18 ± 0·2
NR cage 3	16·0 ± 2·5*	7·71 ± 0·41	5·28 ± 0·56	25·6 ± 4·1	1·68 ± 0·21*
Pooled cages	17·6 ± 1·8*	7·96 ± 0·13	4·99 ± 0·40	25·5 ± 1·3*	1·59 ± 0·22*
BC96 cage 1	22·4 ± 4·0	7·44 ± 0·53	6·22 ± 0·72	21·2 ± 4·6	1·00 ± 0·22
BC96 cage 2	29·5 ± 5·8	9·23 ± 1·31	7·38 ± 0·58	29·1 ± 7·5*	1·47 ± 0·73
Pooled cages	26·0 ± 3·6	8·34 ± 0·90	6·80 ± 0·50	25·2 ± 4·0	1·24 ± 0·24*
BC61	27·3 ± 3·50	8·08 ± 0·79	4·30 ± 0·56	25·0 ± 2·1*	0·99 ± 0·17*

Values are means ± SEM ($n = 5$ for individual cages, or 10 or 15 for 'all cages'), * $p < 0{\cdot}05$ compared with Faroe; [1]total cytochrome P450 content in pmol mg^{-1} protein; [2]'418-peak' (putative denatured P450) content in arbitrary units mg^{-1} protein; [3]CYP1A-immunopositive protein in arbitrary units mg^{-1} protein; [4]benzo[*a*]pyrene hydroxylase (BPH) activity (predominantly phenol formation) in arbitrary fluorescence units mg^{-1} protein; [5]benzo[*a*]pyrene hydroxylase turnover (BPH activity in arbitrary fluorescence units $pmol^{-1}$ P450).

Table 3. Responses of microsomal MFO system components and activities in digestive gland of *M. edulis* transplanted for 6–8 weeks from Faroe Islands to sites in Faroe Islands, Skagerrak and Kattegat during 1994.

Site and cages	Total P450[1]	'418-peak'[2]	'CYP1A'[3]	BPH[4]	BPH turnover[5]
Faroe cage 1	39·4 ± 3·7	18·4 ± 1·3	28·3 ± 4·5	23·7 ± 6·7	0·60 ± 0·15[d]
Faroe cage 2	52·3 ± 10·2[e]	11·2 ± 0·8[b, f]	27·5 ± 6·9	20·7 ± 16·7[c]	0·33 ± 0·24[g]
Faroe cage 3	38·2 ± 4·8[a]	13·9 ± 1·7	25·3 ± 6·6	31·7 ± 3·5	0·83 ± 0·05
Pooled cages	43·3 ± 4·5	14·7 ± 1·9	27·0 ± 0·9	25·3 ± 3·3	0·59 ± 0·14
NR cage 1	35·7 ± 3·8	17·1 ± 2·0[b]	18·4 ± 3·6	57·0 ± 11·4[c]	1·57 ± 0·20[d]
NR cage 2	39·0 ± 1·9	15·2 ± 0·7	27·9 ± 4·3	43·3 ± 21·7	1·60 ± 0·07
NR cage 3	30·0 ± 2·7[a]	15·9 ± 1·1	27·4 ± 1·3	26·7 ± 4·8	0·93 ± 0·23
Pooled cages	34·9 ± 2·6	16·0 ± 0·6	24·6 ± 3·1	42·3 ± 8·8	1·37 ± 0·22*
OS12 cage 1	33·1 ± 7·5	16·7 ± 2·3[f]	27·4 ± 1·3	22·7 ± 8·6	0·76 ± 0·50
OS12 cage 2	30·2 ± 2·3[e]	14·9 ± 1·8	24·5 ± 2·0	38·0 ± 5·5	1·27 ± 0·22[g]
Pooled cages	31·6 ± 1·5	15·8 ± 0·9	26·0 ± 1·5	30·4 ± 7·7	1·02 ± 0·20

Values are means ± SEM ($n = 3$ for individual cages, or 6 or 9 for 'all cages'); comparison of pooled data for all cages at each site –– * $p < 0{\cdot}05$ compared with Faroe; comparison of individual cages—same letter signify $p < 0{\cdot}05$ compared with specified Faroe cage. [1]Total cytochrome P450 content in pmol mg^{-1} protein; [2]'418-peak' (putative denatured P450) content in arbitrary units mg^{-1} protein; [3]CYP1A-immunopositive protein in arbitrary units mg^{-1} protein; [4]benzo[*a*]pyrene hydroxylase activity (BPH) (predominantly phenol formation) in arbitrary fluorescence units mg^{-1} protein; [5]benzo[*a*]pyrene hydroxylase turnover (BPH activity in arbitrary fluorescence units $pmol^{-1}$ P450).

which meet in this area (Magnusson *et al.* 1996). The latter current originates from the German Bight of the North Sea which is known to have higher levels of organochlorines than the central and western parts (Knickmeyer and Steinhart 1988, 1989). Fine-grain material from the North Sea, possibly containing adsorbed contaminants, is deposited in the deep part of the Skagerrak (Stevens *et al.* 1996). Possible local sources of contaminants include the rivers Göta älv (outside

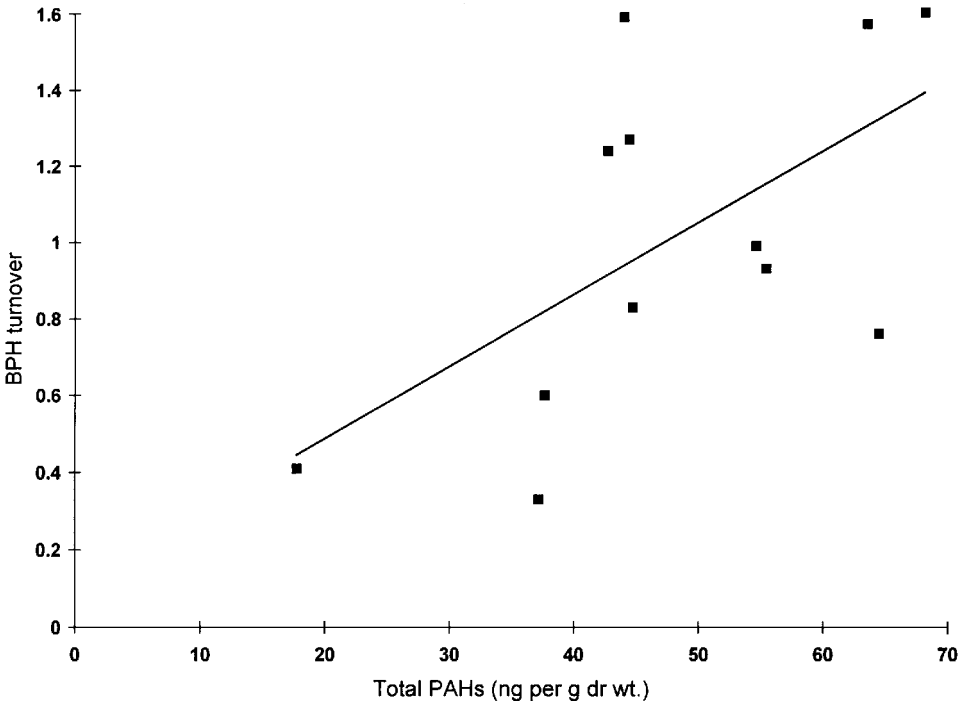

Figure 2. Relationship between benzo[*a*]pyrene hydroxylase (BPH) turnover (i.e. activity per amount total cytochrome P450) of digestive gland microsomes and whole body concentration of total polycyclic aromatic hydrocarbons (PAHs) in *M. edulis* transplanted for 6–8 weeks in the Faroe Islands, Skagerrak and Kattegat (data: mean values for from Tables 1–3); corr. coeff. of regression equation = 0.58.

Göteborg, Sweden) and Glomma (Hvaler Archipelago, Norway) (see Introduction). Bioavailable sediment-bound contaminants have been indicated for several regions of the Skagerrak and Kattegat, including the Göta älv estuary and all the sites examined in this study (Dave and Nilsson 1994, Magnusson *et al.* 1996).

The Skagerrak region of the North Sea has been studied for several years using *M. edulis* as sentinel organisms for pollution studies (Magnusson *et al.* 1988, 1996, Carlberg *et al.* 1989, Cato and Karlsoon 1997). PAHs and PCBs have been the most studied contaminants because of the former's high bioaccumulation by molluscs and the latter's persistence and bioaccumulation along food chains (Varanasi 1989, Walker and Livingstone 1992, Livingstone 1993). Other organochlorinated compounds tend to be less abundant in biota because of their lower tendency to bioacumulate (HCHs, HCB and chlordanes), or their discontinued use (DDT). Although the levels of total PAHs in this study were 57–206 % higher in *M. edulis* transplanted to the Skagerrak and Kattegat compared with the Faroe Islands, on a wider geographical scale the general level of contamination of the former sites corresponds to low level chronic contamination and of the latter to relatively clean conditions. Thus, the highest bioaccumulated total PAH body burden (sum of 2–5 or 3–5 ring PAHs in mg g^{-1} dry wt) of 0·06 for site NR represents about 0·04–20 % of the normal range reported for various coastal and inland waters in Europe and USA (see Livingstone 1991). This lower bioacummulation must also consider that

Table 4. Responses of glutathione S-transferase and antioxidant enzyme activities in digestive gland of *M. edulis* transplanted for 6–8 weeks from Faroe Islands to sites in Faroe Islands, Skagerrak and Kattegat during 1993.

Site	Glutathione S-transferase[1]	Superoxide dismutase[2]	Catalase[3]
Faroe	2·66 ± 0·16	838 ± 80	3·51 ± 0·26
NR	2·85 ± 0·09	616 ± 56	3·85 ± 0·23
BC96	2·79 ± 0·10	623 ± 10	3·26 ± 0·33
BC61	2·02 ± 0·20*	508 ± 102*	2·73 ± 0·24

Values are means ± SEM (n = 5–15), pooled data for all cages for each site only shown (see table 2 for number of cages), * $p < 0{\cdot}05$ compared with Faroe; [1]nmol min^{-1} g^{-1} wet wt; [2]SOD units g^{-1} wet wt; [3]mmol min^{-1} g^{-1} wet wt.

mussels were exposed to pollutants for a short period of time. Similarly, the levels of total body burden PCBs (7–11 ng g^{-1} dry wt) (expressed as sum of the seven congeners, as recommended by ICES for assessing pollution (Duinker *et al.* 1988) are similar to those for clean areas of the Mediterranean coast (Porte and Albaigés 1993), whereas the lower levels of the other organochlorinated compounds (1–5 ng g^{-1} dry wt) are indicative of a relatively non-pesticide contaminated area (Solé *et al.* 1994). The specific activities and contents of the protection enzymes measured in the digestive gland of the transplanted *M. edulis* were similar to those reported in other field studies on *Mytilus* sp. from different parts of Europe (Livingstone 1988, Porte *et al.* 1991, Livingstone *et al.* 1995, Solé *et al.* 1995a, b, Fitzpatrick *et al.* 1997). The higher levels of total cytochrome P450 content, '418-peak' and BPH activity in 1994 compared with 1993 is probably related to the higher contaminant levels encountered in the 1994 survey.

Laboratory and field studies have indicated the existence of an inducible cytochrome P450 monooxygenase system in digestive gland of *Mytilus* sp. and other molluscs, with similarities to vertebrate CYP1A, including the observations of elevation of BPH activity and CYP1A-immunopositive protein with exposure to PAHs and PCBs (see Introduction). Previous field studies on bivalve and gastropod molluscs have shown increases in total cytochrome P450 digestive gland (Porte *et al.* 1991, Yawetz *et al.* 1992, Solé *et al.* 1995a, b), '418-peak' (Livingstone 1988), BPH (Narbonne *et al.* 1991, Michel *et al.* 1994) and/or CYP1A-immunopositive protein (Livingstone *et al.* 1995, Solé *et al.* 1996) with contaminant exposure, but as yet no single parameter has been widely adopted as a biomarker for organic pollution in molluscs (Livingstone 1996, Livingstone and Goldfarb 1997). The elevated BPH activities at the contaminated sites in the Skagerrak, near the Hvaler Archipelago and Göteborg are consistent with observations for *M. galloprovincialis* in the Mediterranean region exposed to similar levels of sediment PAHs (sum of 11 or 12 PAHs), viz. maximal 2·4-fold BPH activity increase at sediment PAHs of up to 3·0 mg g^{-1} dry wt (Magnusson *et al.* 1996) for this study compared with about 3-fold increase in total microsomal BaP metabolism at sediment PAHs of up to 10 in mg g^{-1} dry wt for the Mediterranean study (Narbonne *et al.* 1991). The greater increase in BPH turnover compared with BPH activity with PAH exposure (respectively, maximal 4·7-fold compared with 2·4-fold) is indicative of the induction of specific forms of cytochrome P450, although no increase was detected in CYP1A-immunopositive protein (see below). Greater increase of BPH turnover compared with BPH activity was also seen in

pyloric caeca of the starfish *Asterias rubens* with experimental exposure to BaP (Den Besten *et al.* 1993).

The lack of increase in total cytochrome P450 or '418-peak' contents with contaminant exposure contrasts with elevations in *M. edulis* in a Norwegian fjord (Livingstone 1988) and off the Catalan coast of Spain (Porte *et al.* 1991, Solé *et al.* 1995a, b) exposed to chronic pollution, and in *M. edulis* (Solé *et al.* 1996), the cockle *Cerastoderma edule* (Moore *et al.* 1987), the bivalve *Donax trunculus* and the gastropod *Avicularia gibbosula* (Yawetz *et al.* 1992) following acute exposure to oil spills. The marked lower level of total cytochrome P450 at the Skagerrak NR site in 1993 may be indicative of a stress effect related to depth (650 m), but equally well the decrease was much less marked in 1994, and slight decreases were also indicated at the shallower sites. Elevation of a characteristic 48 kDa microsomal protein recognized by antibody to fish (*P. fluviatilis*) CYP1A was also not seen at the contaminated sites, in contrast to increases observed in digestive gland of *Mytilus* sp. with field exposure to an oil spill off the Galician coast of Spain (Solé *et al.* 1996), or mixed-contaminants (PAHs, PCBs, organochlorines) in the Venice Lagoon, Italy (Livingstone *et al.* 1995, Livingstone 1996), and laboratory exposure to PAHs (Canova *et al.* 1997) and PCBs (Livingstone *et al.* 1997). The extent to which the fish-derived polyclonal antibody recognizes a single or several CYP forms is unknown, but a recent transplant study of *M. galloprovincialis* from a clean to a contaminated site for 3 weeks showed increase in CYP1A-immunopositive protein, but not other proteins recognized by antibodies to mammalian and/or fish CYP2B, CYP2E, CYP3A and CYP4A (Peters *et al.* 1997). Several factors may be involved in the lack of observed increase in CYP1A-immunopositive protein in this compared with other studies, including antibody specificity and different contaminant mixtures, but one may be the lower level of PAH-exposure in the Skagerrak. Thus, the maximal whole tissue level of total PAHs in ng g^{-1} dry wt was 62 for the Skagerrak compared with 307 for the Spanish oil spill (Solé *et al.* 1996) and 504 for the Venice Lagoon (Livingstone *et al.* 1995). Similarly, maximal whole tissue levels of the putative inducer BaP in ng g^{-1} dry wt were 3·8 for the Skagerrak (Förlin *et al.* 1996b) compared with 10 for the Spanish oil spill (Solé *et al.* 1996) and 100–1000 for the laboratory exposure (Canova *et al.* 1997). Seasonal variations in the response of the cytochrome P450 monooxygenase system to contaminants in *Mytilus* sp. have also been observed (Livingstone 1987, Nasci *et al.* 1989, Narbonne *et al.* 1991).

Multiple forms of GST have been characterized in digestive gland of *Mytilus* sp. (Fitzpatrick and Sheehan 1993, Fitzpatrick *et al.* 1997) and its use as a possible biomarker of organic pollution has been proposed (Sheehan *et al.* 1991). However, experimental results with bivalve molluscs have been variable (Livingstone 1991, Michel *et al.* 1993, Regnoli and Principato 1995, Looise *et al.* 1996), and both increases (Kurelec and Pivcevic 1989, Sheehan *et al.* 1991, Rodríguez-Ariza *et al.* 1992, 1993) and no response (Suteau *et al.* 1988, Fitzpatrick *et al.* 1995, 1997, Livingstone *et al.* 1995) in GST activity have been reported with exposure to contaminants in the field. Induction of antioxidant enzymes in response to contaminant-mediated reactive oxygen species (ROS) production and oxidative stress has also been proposed as a possible biomarker of pollution in aquatic organisms, including molluscs (Huggett *et al.* 1992, Lemaire and Livingstone 1993). However, again, responses in bivalve molluscs have been variable, with correlations between elevated SOD and catalase activities and tissue PAHs being

seen in digestive gland of *Mytilus* sp. in field studies in Spain (Porte *et al.* 1991, Solé *et al.* 1995a), but not in the Venice Lagoon, Italy (Livingstone *et al.* 1995). Increases in catalase activity in digestive gland of *M. edulis* have also been seen with exposure to the water-accommodated fraction of crude oil (Cajaraville *et al.* 1992). The lack of marked differences in GST and antioxidant enzyme activities between sites is consistent with the above picture and may be related to such factors as transient antioxidant enzyme responses in aquatic organisms (Lemaire and Livingstone 1993) and seasonal variability in such enzyme activities in *Mytilus* sp. (Viarengo *et al.* 1991, Solé *et al.* 1995b, Power and Sheehan 1996). However, the lower GST and antioxidant enzyme activities seen, or indicated, at the Skagerrak compared with the Faroe Islands site may be indicative of a depth effect.

In conclusion, the 2-year study using transplanted *M. edulis* to study the presence, bioavailability and effects of organic contaminants in the Skagerrak and Kattegat regions of the North Sea has indicated PAH-uptake leading to the putative induction of digestive gland cytochrome P450-dependent BPH activity. This is consistent with other studies in the region which have indicated greater bioavailability of PAHs than organochlorines in the sediments and induction of hepatic CYP1A in the deep-sea fish *Coryphaenoides rupestris* (Förlin *et al.* 1996a). The elevation of BPH turnover in *M. edulis* at the sites outside the Oslo Fjord and Göteborg support the suggestion that the Glomma (Hvaler Archipelago, Norway) and Göta älv (Göteborg, Sweden) estuaries are entry routes of pollution into the Skagerrak (Goksøyr *et al.* 1991, Förlin and Celander 1993). The lack of increase in total cytochrome P450 content and CYP1A-immunopositive protein levels at the contaminated sites argues for a multiparameter approach until more is known of the characteristics of the molluscan cytochrome P450 monooxygenase system and/or specific molluscan antibodies become available. The likely consequences of PAH-uptake and elevated BPH activity could include damage to DNA via ROS formation and adduct formation (Garcia Martinez and Livingstone 1995, Canova *et al.* 1997, Sjölin and Livingstone 1997). The general lack of induction of GST and antioxidant enzyme activities confirms the limited use of these biomarkers in environmental monitoring.

Acknowledgements

The work was carried out under the tenure of a Spanish postdoctoral fellowship from the 'Consejo Superior de Investigaciones Científicas' (CSIC) to M. Solé, a CEC grant award (BIOMAR-No. EV5V-CT94-0550) to D.R. Livingstone, and support by the Swedish Environmental Protection Agency.

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