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Study of the biological impact of organic contaminants on mussels (*Mytilus galloprovincialis* L.) from the Venice Lagoon, Italy: responses of CYP1A-immunopositive protein and benzo(*a*)pyrene hydroxylase activity

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A survey to evaluate the impact of organic contaminants on the mussel *Mytilus galloprovincialis* in the Venice Lagoon, Italy was carried out in May 1993. *M. galloprovincialis* were sampled from putative moderately contaminated (Alberoni, Lio Grande, Crevan), urban (Salute) and industrial (CVE) sites in the Venice Lagoon, and from a clean reference site (Plataforma) in the adjacent Adriatic Sea. Measurements comprised (i) whole-tissue body burdens of aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and other organochlorines (DDTs, hexachlorocyclohexanes and hexachlorobenzene); and (ii) digestive gland microsomal cytochrome P450 (CYP)-dependent monooxygenase system (i.e. total CYP and cytochrome P4501A (CYP1A)-immunopositive protein levels, benzo(*a*)pyrene hydroxylase (BPH) activity) as a specific biomarker of impact by organic contaminants. Chemical analysis identified a contaminant gradient with Plataforma as the cleanest and CVE followed by Salute as the most contaminated extremes. No elevation of total CYP content or CYP1A-immunopositive protein level was seen at any of the lagoon sites compared with Plataforma. In contrast, BPH activity and BPH turnover (i.e. BPH activity per amount total CYP) were respectively 1- and 2.5-fold higher at CVE than Plataforma ($P < 0.05$), and indicated to be higher (up to 1-fold) at all the other lagoon sites compared with Plataforma. Correlation was seen between BPH activity and tissue levels of total aliphatic hydrocarbons ($r = 0.94 – 0.98$), but not between the former and total PAHs or PCBs. The results are consistent with other studies in the area and indicate greatest biological impact of contaminants was at CVE followed by the other lagoon sites, with a possible genotoxic role for the elevated BPH activity in the formation of bulky DNA-adducts.

Keywords: PAHs, *Mytilus galloprovincialis*, cytochrome P450 monooxygenase system, benzo(*a*)pyrene hydroxylase, Venice Lagoon, Adriatic Sea.

Abbreviations: BaP, benzo(*a*)pyrene; BPH, benzo(*a*)pyrene hydroxylase; CYP, cytochrome P450; CYP1A, cytochrome P4501A; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; IgG, immunoglobulin G; MFO, mixed-function oxygenase; NADPH, β–nicotinamide adenine dinucleotide phosphate reduced form; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorobiphenyl; UCM, unresolved complex mixture.

Introduction

There is little doubt of the presence of toxic chemicals in the 550 km^2 area of the Venice Lagoon, Italy due mainly to the existence of chemical plants and oil refineries situated on the mainland in Porto Marghera (figure 1; Nasci *et al*. 1989, Zatta *et al*. 1992, Fossato *et al*. 1999). In order to understand the magnitude and implications of the problem, the presence and effects of contaminants in the Venice Lagoon has been the subject of a range of studies carried out under the UNESCO

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Venice Lagoon Ecosystem Project. The aim of the UNESCO project has been to assess not only the environmental health of the region, but also the quality of its waters which support an important shellfish industry. An estimated annual production of about 2500 tons of mussel (*Mytilus galloprovincialis* L.) represents a high economic interest for the region. Besides their economic value, sedentary filter-feeding mussels are also used worldwide as sentinel organisms for monitoring pollution in aquatic ecosystems (Goldberg *et al.* 1978), their low rates of biotransformation relative to rates of uptake resulting in marked bioaccumulation of organic contaminants, which in turn reflect environmental exposure levels (Livingstone 1991, 1998, Walker and Livingstone 1992). In common with other organisms, they also possess the ability to respond at the molecular, cellular and higher-order levels of biological organization to the presence of contaminants (Livingstone 1996, Livingstone and Goldfarb 1998, Livingstone *et al.* 1999). The integrated measurement of such biological responses and/or effects (biomarkers), together with tissue contaminant levels, are being increasingly used in pollution monitoring, both as early warnings of exposure and as aids to impact assessment in the aquatic environment (Huggett *et al.* 1992, Schlenk 1996, Walker *et al.* 1996, Livingstone *et al.* 1999).

Interest in monitoring the degree of pollution for petrogenic compounds in the Venice region using *M. galloprovincialis* as sentinel species goes back to the early 1970s (Fossato and Siviero 1974), but soon after chlorinated hydrocarbons (Fossato and Craboledda 1979) and metals (Fossato *et al.* 1999, Zatta *et al.* 1992) were also measured. A comprehensive review on the presence and trends of persistent contaminants in the biota of the Venice Lagoon ecosystem has been reported in Fossato *et al.* (1999). In more recent years, biological responses and effects have also been incorporated into the monitoring and impact assessment of the region, the former including investigations at the biochemical (Nasci *et al.* 1989, Livingstone *et al.* 1995, Wootton *et al.* 1995, Livingstone and Nasci 1999), genotoxicological (Venier *et al.* 1996), cellular (Lowe *et al.* 1995) and immunological (Pipe *et al.* 1995) levels in *M. galloprovincialis* and/or fish.

The major aim of the study of this paper was to assess the apparent responses of the cytochrome P450 (CYP)-dependent monooxygenase system of the digestive gland of *M. galloprovincialis* in relation to its tissue body burdens of organic contaminants. Measurements of the latter comprised aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs) and organochlorinated pesticides such as DDTs and hexachlorohexanes, many of which are priority contaminants in pollution monitoring programmes worldwide (Duinker *et al.* 1988, Walker and Livingstone 1992). Certain PAHs are of particular interest due to their activation to mutagenic and carcinogenic metabolites by the CYP-dependent monooxygenase system (Walker and Livingstone 1992). The CYP-dependent monooxygenase or mixed-function oxygenase (MFO) system is of central importance in the biotransformation of many organic contaminants, including PAHs and PCBs. Induction of the CYP isoform cytochrome P4501A (CYP1A) is used routinely worldwide in liver and other tissues of fish as a specific biomarker of exposure to PAHs, PCBs and other organic contaminants (Bucheli and Fent 1995, Livingstone 1996, Livingstone and Goldfarb 1998). Although much less is known of these processes in molluscs, recent laboratory (Livingstone *et al.* 1997, Canova *et al.* 1998) and field (Livingstone *et al.* 1995, Solé *et al.* 1996, 1998, Wootton *et al.* 1996, Peters *et al.* 1998a, 1999) studies have indicated the

existence of an MFO system in the digestive gland of mytilid bivalves inducible by PAHs and PCBs, including a microsomal protein recognized by antibodies to fish hepatic CYP1A which is involved in the metabolism of benzo(*a*)pyrene (BaP) to phenols.

In this study, *M. galloprovincialis* were collected from five stations in the Venice Lagoon exposed to industrial and domestic pollution, and from a relatively clean reference station in the adjacent Adriatic Sea. Apparent responses of the MFO system of digestive gland microsomes of *M. galloprovincialis* were assessed in terms of total CYP content, CYP1A-immunopositive protein level and BaP hydroxylase (BPH—metabolism of BaP to phenols) activity. The results are discussed in relation to other studies in the Venice Lagoon and to the current state of utility of the mytilid MFO system as a specific biomarker of organic pollution.

Materials and methods

Animals and tissue collection

M. galloprovincialis (4–6 cm shell length) of mixed-sex were collected in May 1993 from the Adriatic Sea (site: Plataforma) and from several sites in the Venice Lagoon: Alberoni and Lio Grande (north of Venice), Crevan (south of Venice), CVE (industrial site) and Salute (urban site) close to the city of Venice (Central Lagoon) (figure 1). Mussels were immediately dissected. Whole tissues were stored at –20°C for contaminant analysis, while digestive glands from other individuals were damp-dried, frozen in liquid nitrogen and stored at –75°C prior to transportation on dry ice to Plymouth for biochemical analysis.

Chemicals

Biochemicals, including β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH), BaP, goat anti-rabbit IgG (whole molecule) alkaline phosphatase conjugate 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 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

Chemical analyses were carried out at the CNR Istituto di Biologia del Mare, Venice. Pooled whole tissue samples (10–15 specimens of mussels per sample) were lyophilized, blended and their wet/dry weight recorded. For hydrocarbons and chlorinated compounds analysis, lyophilized blended samples were subjected to Soxhlet extraction with *n*-hexane and further clean-up by alumina/silica gel chromatography. Chromatographic conditions, detailed procedures and characteristics of the internal standards are described elsewhere (Fossato *et al*. 1999; see also Lowe *et al*. 1995, Pipe *et al*. 1995). Aliphatic hydrocarbons are seen in the gas chromatograms as a series of resolved peaks (*n*-alkanes) and a 'hump' made of a series of non-resolved compounds, unknown complex mixture (UCM). Quantification of *n*-alkanes ranged from *n*-C_{15} to *n*-C_{30} and these were analysed by gas chromatography (GC) equipped with a flame-ionization detector. Organochlorines, including PCBs, HCHs and HCB, were analysed by GC equipped with an electron-capture detector, and PAHs by high performance liquid chromatography with 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 site. Microsomal fractions were prepared at 4°C by differential centrifugation, essentially as described previously (Solé *et al*. 1998). 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 KCl, 0.5 M sucrose. Microsomal fractions were obtained in 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.

All assays were carried out in duplicate. BPH activity was measured at 25°C and was linear with time and about a five-fold range of sample concentration. MFO components and BPH activity were measured on microsomes as follows. Total CYP and '418-peak' (putative denatured CYP—see

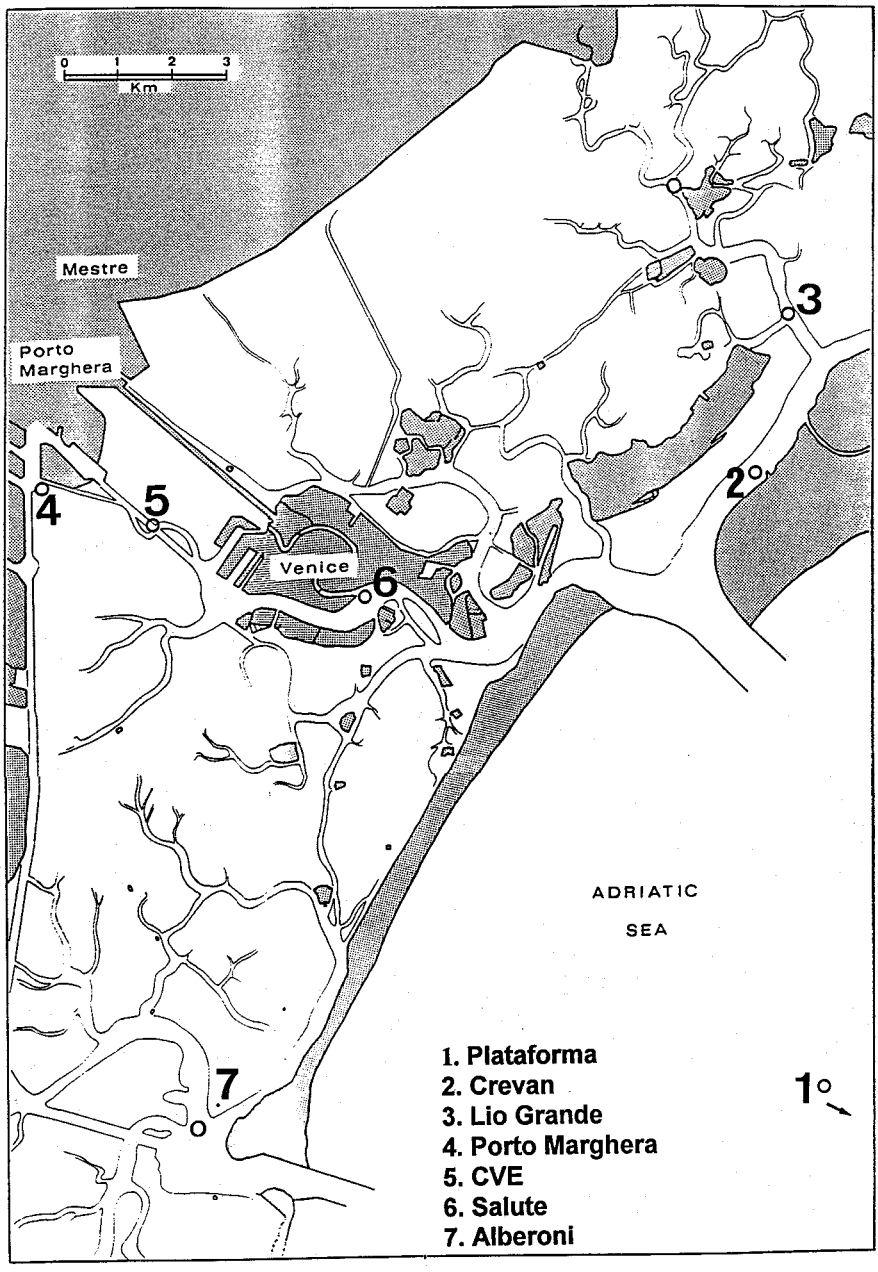

Figure 1. Map of the Venice Lagoon, Italy. Sampling sites for mussels and industrial Porto Marghera are indicated. Coordinates are for longitude E between 12°10′ and 12°35′ and for latitude N between 45°10′ and 45°35′.

Livingstone 1991) contents were assayed by the carbon-monoxide difference spectrum of sodium dithionite-reduced sample as described in Livingstone (1988) using an extinction coefficient of 91 mM^{-1} cm^{-1} for CYP and arbitrary units for the '418-peak'. 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 CYP from digestive gland of *M. edulis* (Porte *et al.* 1995) and hepatic microsomes from β-naphthoflavone-induced turbot (*Scophthalmus maximus*) (Peters and

Livingstone 1995). BPH 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 mM 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 addition of 1 ml cold acetone. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical treatment

The biochemical results are presented as mean±SEM ($n = 5$). Differences between groups of values were tested by multivariance one way ANOVA analysis, $P < 0.05$ was accepted as statistically significant.

Results

The levels of organic contaminants measured in single samples of pooled whole tissues of *M. galloprovincialis* are given in table 1. Differences between sites are evident, with Plataforma in the Adriatic Sea indicated to be the cleanest site, and industrial CVE and urban Salute the most polluted sites, with respect to most contaminants. The other sites of Lio Grande, Alberoni and Crevan generally had intermediate contaminant body burdens. Total *n*-alkanes and UCM both ranged about five-fold between sites, and respectively were highest at CVE compared with Plataforma and Plataforma or Alberoni. Total PAHs ranged 5.5-fold and were 4.5-fold and 2.3-fold higher in Salute and CVE, respectively, compared with Plataforma. Total PCB levels showed the greatest gradient of difference between sites (12.7-fold range), and were about an order of magnitude higher in both Salute and CVE compared with the Adriatic Sea Plataforma site. About five-fold ranges were seen for both total DDTs and HCHs, which were highest in both Salute and CVE compared with the other sites. A common petrogenic source of aliphatic compounds (*n*-alkanes and UCM) is possibly indicated from the high correlation between the two groups of chemicals at the different sites ($r = 0.96$). Similarly, a common origin of PAHs and PCBs is also possibly indicated by their high covariance between sites ($r = 0.93$).

The results for MFO components and BPH activity in digestive gland microsomes of *M. galloprovincialis* from the different sites are presented in table 2. Total CYP content was up to 1.2-fold higher in mussels from the Adriatic Sea site

Table 1. Organic contaminant levels in whole soft tissues of mussel *M. galloprovincialis* from sites in the Venice Lagoon, Italy and in the Adriatic Sea (Plataforma).

Site	*n*-alkanes[a]	UCM[a]	PAHs[b]	PCBs[b]	DDTs[b]	HCHs[b]
CVE	7.2	575	299	322	23.0	5.1
Salute	3.0	323	504	406	34.6	3.4
Lio Grande	2.1	242	229	101	4.5	1.5
Alberoni	1.9	98	209	74	7.3	1.0
Crevan	1.8	170	123	53	9.5	1.5
Plataforma	1.4	102	91	34	6.2	2.3

UCM, unresolved mixture; PAHs, total polycyclic aromatic hydrocarbons; PCBs, total polychlorobiphenyls; DDTs, total 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT) and metabolites 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE); HCHs, total hexachlorocychlohexanes (for other details see Fossato *et al.* 1999).

[a] µg g^{-1} dry weight.

[b] ng g^{-1} dry weight.

Table 2. Mixed-function oxygenase system components and benzo(*a*)pyrene hydroxylase activity in digestive gland of mussel *M. galloprovincialis* from sites in the Venice Lagoon, Italy and in the Adriatic Sea (Plataforma).

Site	CYP[a]	'418-peak'[b]	'CYP1A'[c]	BPH[d]	BPH/CYP[e]
CVE	26.6 ± 5.1	10.4 ± 1.7	40.7 ± 3.5	82.2 ± 9.6*	3.5 ± 0.5*
Salute	27.2 ± 3.3*	11.1 ± 1.2	43.3 ± 2.1	51.2 ± 12.2	1.9 ± 0.4
Lio Grande	27.1 ± 2.5*	8.2 ± 1.2*	39.4 ± 2.4	50.6 ± 8.8	1.9 ± 0.4
Alberoni	24.1 ± 3.2*	8.2 ± 0.8*	39.1 ± 1.6	45.4 ± 7.1	2.1 ± 0.5
Crevan	30.1 ± 5.7*	11.4 ± 1.0	34.7 ± 1.4	51.1 ± 10.2	1.8 ± 0.4
Plataforma	57.5 ± 10.4	12.0 ± 1.4	49.6 ± 2.9	43.4 ± 10.7	1.0 ± 0.5

CYP, total cytochrome P450; CYP1A, CYP1A-immunopositive protein; BPH, benzo(*a*)pyrene hydroxylase activity; BPH/CYP, turnover of benzo(*a*)pyrene hydroxylation per amount CYP; values are means ± SEM ($n = 5$).

[a] pmol mg^{-1} microsomal protein.

[b] Arbitrary units mg^{-1} microsomal protein.

[c] Arbitrary units mg^{-1}.

[d] Arbitrary fluorescence units mg^{-1} microsomal protein.

[e] Arbitrary units $pmol^{-1}$ CYP.

* $P < 0.05$ compared with Plataforma.

compared with the Venice Lagoon site which all showed similar total CYP contents (26.6–30.1 with an overall mean of 27.0 compared with 57.5 pmol mg^{-1} for Plataforma). Highest levels of '418-peak' were also indicated at Plataforma, but the differences were only statistically significant compared with the Lio Grande and Alberoni sites ($P < 0.05$). Levels of CYP1A-immunopositive protein varied relatively little between all sites, being highest at Plataforma, but no differences were statistically significant. However, a degree of correlation was observed between total CYP and CYP1A-immunopositive protein across the sites ($r = 0.78$). BPH activity was indicated to be highest at the industrial CVE site and lowest at the Plataforma reference site, the former being significantly ($P < 0.05$) about one-fold higher than the latter. The differences between sites were greatest when BPH activity was expressed in terms of per amount total CYP (i.e. BPH turnover) rather than per amount microsomal protein. In this case, a gradient of difference was indicated, with BPH turnover for CVE being 2.5-fold higher than for Plataforma, and indicated to be about one-fold higher than for Salute, Lio Grande, Alberoni and Crevan. Comparing digestive gland microsomal BPH activity and BPH turnover with total tissue contaminant levels, a high correlation was seen between both biochemical measurements and aliphatic hydrocarbons, viz. BPH, $r = 0.98$ and 0.94 (respectively, for *n*-alkanes and UCM) and BPH turnover, $r = 0.92$ and 0.86 (respectively, for *n*-alkanes and UCM). In contrast, no correlation was evident between BPH activity or BPH turnover and total PAHs ($r = 0.30$).

Discussion

The degree of chemical pollution in the waters around Venice is well-documented, and a compilation of organic and metal contaminant levels in tissues of *M. galloprovincialis* from 1976 to 1993 has recently been reported (Fossato *et al.* 1999). Past and recent analyses over this 17-year period have consistently identified the industrial CVE and urban Salute sites as the most heavily contaminated areas in the Venice Lagoon, with highest contaminant body burdens in the biota, including

M. galloprovincialis, compared with the other sites inside (Crevan, Alberoni, Lio Grande) and outside (Plataforma) the lagoon (Livingstone *et al*. 1995, Lowe *et al*. 1995, Fossato *et al*. 1990; this paper—table 1). From 1976 to 1993, contaminant body burdens in *M. galloprovincialis* showed trends of decreasing levels of the organochlorine pesticides HCHs and DDTs, a steady situation for PCBs and most trace metals, and an increase in petroleum hydrocarbons at some sites, including CVE and Salute (Fossato *et al*. 1999). A similar trend of decreasing levels of some organochlorinated compounds, plus a change in PCB congener composition, has also been reported in *Mytilus* sp. in the north-western Mediterranean region (Solé *et al*. 1994). The contaminant levels in tissues of *M. galloprovincialis* from the Venice Lagoon are in the range of those obtained in *Mytilus* sp. in other areas of the Mediterranean region (Porte and Albaigés 1993, Picer and Picer 1995, Baumard *et al*. 1998).

Putative induction of the digestive gland microsomal MFO system of *Mytilus* sp. has been used in a variety of field situations in recent years as a specific biomarker of exposure to PAHs, PCBs and other organic contaminants, although some inconsistencies have been seen in the apparent responses of the major parameters measured (total CYP, CYP1A-immunopositive protein, BPH) (Livingstone 1996, Livingstone and Goldfarb 1998, Peters *et al*. 1998a, 1999, Solé *et al*. 1998). Inconsistency in the apparent response of the digestive gland MFO system of *M. galloprovincialis* in relation to the contaminant body-burdens was also seen in this study, with the higher BPH activity at CVE not being paralleled by elevated total CYP or CYP1A-immunopositive protein. No elevation of total CYP content was observed at the more contaminated sites of CVE and Salute, or at any of the Venice Lagoon sites, compared with Plataforma in the Adriatic Sea. On the contrary, *M. galloprovincialis* from the cleaner Plataforma site displayed higher total CYP content than the lagoon sites. However, this result was in contrast to that for the previous year of 1992 which showed similar digestive gland microsomal total CYP contents in *M. galloprovincialis* at all of the sites examined (CVE, Salute, Lio Grande, Crevan, Plataforma) (Livingstone *et al*. 1995, Livingstone and Nasci 1999). Higher digestive gland microsomal total CYP content at clean (the Faroe Islands) compared with contaminated (Skagerrak and Kattegat, the North Sea) sites was also seen in a study with transplanted *M. edulis* (Solé *et al*. 1998). Evidence linking contaminant exposure and an increase in total CYP content of digestive gland of bivalves has been seen in several other field studies, including long-term exposure to industrial and urban contaminants (*M. galloprovincialis*—Porte *et al*. 1991, Solé *et al*. 1995a), a situation similar to that of the current study. However, in most cases, the observed increases in total CYP content were in response to short-term oil spills in mesocosms (*M. edulis*—Livingstone 1988), or the field (*M. edulis*—Solé *et al*. 1996; *Cerastoderma edule*—Moore *et al*. 1987; *Donax trunculus*—Yawetz *et al*. 1992). Other factors which could possibly operate over the long-term to deplete levels of CYP and other proteins in chronically-contaminated situations include reduced energy balance (Widdows and Donkin 1992) and inhibition of protein synthesis as is seen for the effects of cobalt on mammalian CYP (Timbrell 1991). The latter metal was elevated about two- to four-fold in 1993 in *M. galloprovincialis* at the Venice Lagoon sites compared with Plataforma (Lowe *et al*. 1995). The less marked differences in levels of digestive gland microsomal '418-peak' (putative denatured CYP) between sites were also seen in the study of 1992 (Livingstone *et al*. 1995). In other field studies, both no change (Solé *et al*.

1998) and increases (Moore *et al*. 1987, Livingstone 1988) in levels of '418-peak' with contaminant exposure have been seen in bivalves.

Similar to total CYP content, an indication of elevated levels of digestive gland microsomal CYP1A-immunopositive protein at the cleaner Plataforma site compared with the Venice Lagoon sites was observed, although it did not reach statistical significance ($P > 0.05$). No significant correlation was thus found between the levels of CYP1A-immunopositive protein and measured tissue contaminants, despite such relationships being observed, or indicated, in some previous field studies (Solé *et al*. 1996), including Venice Lagoon in 1992 (CYP1A-immunopositive protein levels were ~one-fold higher at CVE than Plataforma) (Livingstone *et al*. 1995), or after laboratory exposure to BaP (Canova *et al*. 1998) or PCBs (Livingstone *et al*. 1997). Several factors may be involved in the lack of an observed increase in CYP1A-immunopositive protein with contaminant exposure in this study compared with others, including antibody specificity and field contaminant levels and composition. In a transplant study of *M. galloprovincialis* from a clean site within Venice Lagoon (Punta Lido) to CVE for 3 weeks, increases were observed in levels of digestive gland CYP1A-immunopositive protein, but not in other microsomal CYPs recognized by antibodies to mammalian and/or fish CYP2B, CYP2E, CYP3A and CYP4A (Peters *et al*. 1998a, b), indicating good specificity of recognition of the fish anti-CYP1A antibody for the inducable *M. galloprovincialis* protein. In contrast, good correlations between total CYP content and CYP1A-immunopositive levels in situations of both elevation of the two (Solé *et al*. 1996), and no increase in the two (Solé *et al*. 1998; this paper), argue that the fish anti-CYP1A antibody may be recognizing more than one *M. galloprovincialis* CYP form. Quantitative contaminant considerations shed no light on the lack of increase in digestive gland microsomal CYP1A-immunopositive protein with contaminant exposure in this study. The latter observation contrasts with elevations seen in the 1992 Venice Lagoon study (Livingstone *et al*. 1995) and following an oil spill off the northern Spanish Coast (Solé *et al*. 1996), despite similar whole tissue levels of total PAHs of respectively 504, 443 and 307 ng g^{-1} dry weight in the three studies.

Digestive gland microsomal BPH activity was the only MFO parameter significantly enhanced in *M. galloprovincialis* at the contaminated CVE compared with the Plataforma site, and was indicated to be higher at all the other lagoon sites (Salute, Lio Grande, Alberoni, Crevan) compared with Plataforma. The greater elevation in BPH turnover than BPH activity at CVE compared with Plataforma (i.e. 2.5-fold compared with 1-fold) is indicative of the induction of specific CYP forms, although no increase was detected in CYP1A-immunopositive protein. Similar observations of increase in BPH activity, but not CYP1A-immunopositive protein, with contaminant exposure have also been described in a field transplant study with *M. edulis* exposed to PAHs and other contaminants (Solé *et al*. 1998), and in a laboratory exposure of the starfish *Asterias rubens* to BaP (Den Besten *et al*. 1993). In other field studies, elevation of microsomal BPH activity in *Mytilus* sp. has correlated well with levels of total PAHs in tissues or sediments, viz. in the North Sea (Solé *et al*. 1998) and the French Mediterranean coast (Garrigues *et al*. 1993, Narbonne *et al*. 1991, Michel *et al*. 1994). However, in this study correlation was not observed between BPH activity and tissue levels of total PAHs, but between the former and tissue aliphatic hydrocarbons (*n*-alkanes and UCM; $r = 0.94 - 0.98$). A similar correlation in *M. galloprovincialis* from the same region in 1992 and 1993 was seen, or indicated, between digestive gland putative CYP1A-

mRNA and tissue aliphatic hydrocarbons but not total PAHs (Wootton 1995). However, up to now there are no reports of MFO induction in molluscs by aliphatic hydrocarbons to aid interpretation of these results, and the presence of other unidentified inducing agents cannot be ruled out. The lack of correlation of digestive gland microsomal BPH activity with PAH and organochlorine (HCHs, DDTs, PCBs) was most obvious in urban contaminated Salute compared with the industrial CVE site, the former surprisingly showing no greater levels of BPH activity than the less contaminated other lagoon sites (Lio Grande, Alberoni, Crevan). The lack of correlation between BPH activity and levels of CYP1A-immunopositive protein in this study contrasts with the parallel increase in the two MFO parameters in digestive gland of *Mytilus* sp. following long-term exposure to contaminant gradients in the Mediterranean Sea and shorter-term exposure to an oil spill (Peters *et al*. 1999).

Considering overall the BPH activity and tissue contaminant data of this paper, and the MFO and contaminant results for the region taken at other times (Livingstone *et al*. 1995, Livingstone and Nasci 1999), the findings are consistent with several studies showing contaminant impact at different levels of biological organization in *M. galloprovincialis* which is (i) greater inside the Venice Lagoon than outside in the Adriatic Sea, and (ii) within the lagoon greatest at the industrial CVE site. The observed biological effects include damage to blood cell lysosomes (Lowe *et al*. 1995), altered immune response capability (Pipe *et al*. 1995), and the formation of bulky aromatic DNA-adducts in gills (Venier *et al*. 1996). The latter is consistent with the known presence of CYP in bivalve gills (Livingstone 1996) and the demonstrated involvement of CYP-catalysed reactions in the genotoxicity of BaP in *M. edulis* (Mitchelmore *et al*. 1998). The lower digestive gland microsomal BPH activity at Salute compared with CVE presumably may be due to the characteristics of the different contaminant mixtures present at the two sites from, respectively, domestic sewage compared with industry and heavy boat traffic. However, differences between populations could also be caused by seasonal variations in the mytilid digestive gland MFO system (Suteau *et al*. 1985, Livingstone 1987, Kirchin *et al*. 1992, Solé *et al*. 1995). The toxicity of parent aliphatic hydrocarbons appears to have little impact on such mytilid physiological functions as filtration rate, but could be enhanced by oxidation processes (Thomas *et al*. 1995). Aliphatic hydrocarbons are often present as a result of boat traffic, boat cleaning and industrial activities, whereas PAHs are mostly the result of incineration of organic residues and fuel oil pyrolysis and are usually present in direct relation to the degree of industrial activity (Neff 1979); both are also produced by oil spills. Finally, in terms of the biomarker potential of the mytilid MFO system, field studies have now been reported showing elevation of BPH activity alone (Solé *et al*. 1998; this paper), CYP1A-immunopositive protein level alone (Solé *et al*. 1996), and both BPH activity and CYP1A-immunopositive protein level together (Livingstone *et al*. 1995, Peters *et al*. 1999) with contaminant exposure. Whereas seasonal profiles (Wootton *et al*. 1996) and mechanistic studies (Livingstone *et al*. 1997, Mitchelmore *et al*. 1998) of the digestive gland MFO system have indicated a major involvement of a CYP1A-like enzyme in BaP metabolism, they also indicate that other CYPs could be involved. Robust application of the MFO system as a biomarker will therefore depend on a more complete mechanistic understanding of processes and CYP(s) involved, and the availability of probes specific for these CYP(s).

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