Assessment of a glutathione S-transferase and related proteins in the gill and digestive gland of *Mytilus edulis* (L.), as potential organic pollution biomarkers

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The response of the glutathione S-transferase (GST, EC 2.5.1.18) and related proteins of *Mytilus edulis* to environmental pollution load was assessed. Mussels were reciprocally transplanted between an industrial estuary (Douglas), a rural estuary (Youghal) and a marine site (Bantry). In addition, mussels were sampled along a pollution gradient in an estuary receiving leather tannery effluent (Colligan). These latter mussels were previously shown to be subject to oxidative stress resulting from the discharges. GST specific activity of cytosolic extracts from the gill and digestive gland tissues was determined for all animals. Specific activity was shown to vary significantly in animals from different sites, with highest specific activity always observed in samples (local and transplanted) taken from the industrial site. By comparison, the mussels exposed to tannery discharges displayed no significant alteration in GST specific activity. Total intracellular glutathione (GSH) was also determined for samples taken from the Douglas and Youghal estuaries but no correlation with pollution load was observed. Using FPLC analysis, we observed no specific effect on the relative levels of the GST and the individual GST related proteins in gill or digestive gland samples from local or reciprocally transplanted animals from Douglas or Youghal. The increase in GST specific activity observed in samples from the industrial estuary are indicative of a possible, specific inductive agent at this site. The results from the tannery site, by comparison, indicate that general oxidative stress does not result in elevated GST specific activity in *M. edulis*.

Keywords: *glutathione S-transferase*, Mytilus edulis, *gill, digestive gland, specific activity, oxidative stress.*

Introduction

Some common biochemical and cellular responses which have been used as indices of exposure to environmental contamination include (1) variations of specific enzyme activities such as cytochrome P-450 (Stegeman and Lech 1991, Goksøyr and Förlin 1992), (2) protein levels, e.g. metallothioneins (Kille *et al.* 1992) and stress proteins [initially referred to as heat shock proteins] (Sanders 1993) and (3) lysosomal membrane stability (Moore 1991). Detoxification enzymes are potentially useful in the assessment of the response of an animal to environmental pollution, as these enzymes are usually rapidly induced and sensitive, they may therefore be used to detect the presence of bioactive pollutants.

The detoxification enzymes have been divided into phases I, II, and III, generally reflecting the order in which a xenobiotic is metabolized by them (Ishikawa 1992). The GSTs (glutathione S-transferase, EC 2.5.1.18) are quantitatively the most ubiquitous of the phase II detoxification enzymes. As predominantly cytosolic enzymes, the ease with which the GSTs may be assayed has allowed a detailed study of their distribution. These enzymes catalyse the conjugation of a large variety of xenobiotics as well as endogenous substrates to glutathione (GSH). Almost all organisms investigated possess this mechanism of detoxification (Stenersen *et al.* 1987). Comparisons between organisms may be readily made when the compound 1-chloro-2,4-dinitrobenzene (CDNB) is used as substrate. This compound is very active as a substrate for most GSTs tested. Its use in the screening of organisms for GST activity is likely to give a reasonable estimation of the sum of the catalytic activities of most GSTs present in a preparation (Mannervik and Danielson 1988).

Elevation of molluscan GST activity as a result of exposure to certain compounds has been demonstrated (Boryslawskyj *et al.* 1988). Increases in GST activity have also been demonstrated in other invertebrates such as crabs (Lee *et al.* 1988) and crayfish (Lindström-Seppä and Hänninen 1986). In addition, there is evidence which suggests that GST activity does not vary significantly under the influence of environmental parameters such as sex, age and seasonality (Sheehan *et al.* 1991, Power and Sheehan 1995). These features are very desirable of any parameter to be used as a possible biomarker. In contrast, the cytochrome P-450 isoenzymes have been clearly shown to be influenced by seasonality (Kirchin *et al.* 1992), thereby making interpretation of variations in the levels of these enzymes more difficult. However, pollution correlated differences in P450-related parameters but not GST have been observed in *M. edulis* in certain field studies (Fitzpatrick *et al.* 1995a, Livingstone *et al.* 1995).

In this paper we assess the feasibility of using the GSTs of *M. edulis* as potential biomarkers of chemical pollution load in the environment. GST specific activity was determined in the gill and digestive gland tissues of *M. edulis* from a number of sites of varying pollution load, i.e. (1) Douglas Estuary, adjacent to an area receiving effluents from a number of pharmaceutical plants, (2) Bantry, a pristine marine site, also the location of a commercial mussel farm and (3) Youghal, a relatively unpolluted estuary. In addition, mussels were reciprocally transplanted between the sites to study short term induction. Furthermore, as oxidative stress has previously been shown to induce GST expression in various mammalian cell lines (Daniel 1993), mussels were collected along a pollution gradient in the Colligan Estuary (see Figure 1). These mussels have previously been shown to be subject to oxidative stress resulting from the discharges (Walsh and

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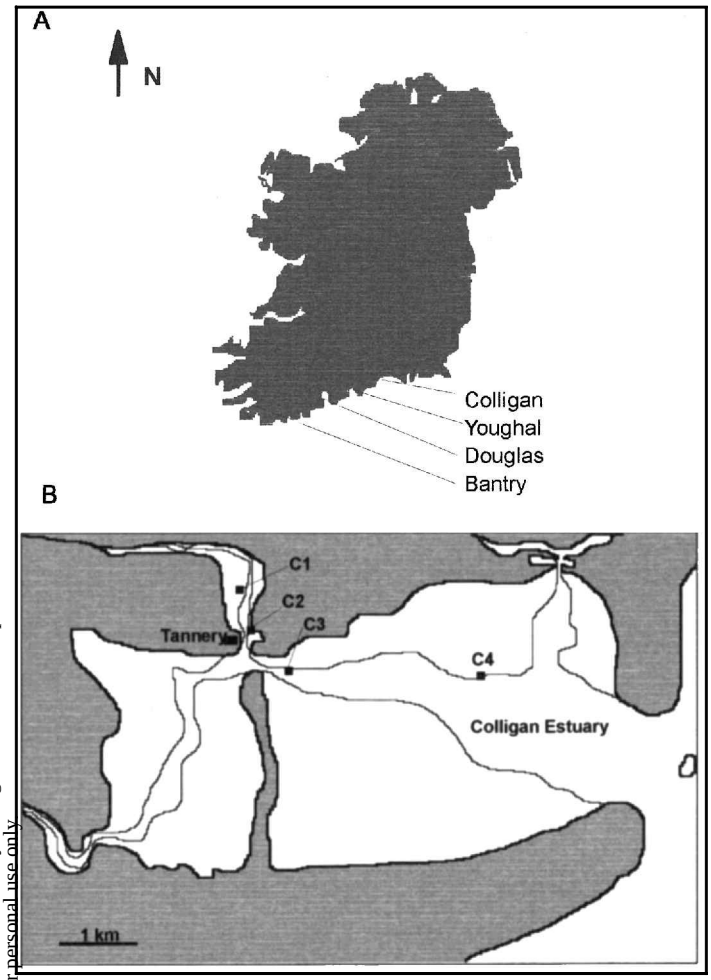

Figure 1. (A) Indicates the location of the areas from which mussels were sampled on the South coast of Ireland. B) Blow up of the Colligan Estuary showing the location of sites 1–4 (C1–C4). Site 1 (Colligan 1) is the most upstream and therefore has the lowest salinity and site 4 (Colligan 4) is nearest the mouth of the estuary and hence has the highest salinity. The shaded areas represent the land around the estuary and the white areas represent the waterbody of the estuary.

O'Halloran 1996). The causative agent was demonstrated to be the aromatic fungicide Busan 30WB containing the active ingredient 2-(thioocyanomethylthio)-benzothiazole (TCMTB). In addition, the pollution gradient also corresponds to a salinity gradient, and as such the effect of salinity on GST specific activity in *M. edulis* was also investigated.

The expression of specific enzyme activity in this study was measured using tissue dry weight as the denominator of expression as both cytosolic protein and tissue wet weight were found to be unsuitable for use under field conditions (Fitzpatrick *et al.* in prep.). This was due to the finding that tissue water and particularly cytosolic protein concentrations varied significantly in the tissues of mussels between sites.

MATERIALS AND METHODS

Mussel transplantation experiments

Mussels were reciprocally transplanted from reference sites to more contaminated sites. Over 200 mussels between 50 and 60 mm in length were collected from each site (see Figure 1). Mussels sampled from Bantry Bay were taken from a rope grown cultivated population, while samples from Douglas, Youghal and Colligan estuaries were collected intertidally. Within 24 h of collection (having been maintained in static 25 l tanks of filtered aerated sea water in the interim), the mussels were placed in polypropylene baskets and distributed to the relevant sites. All baskets were placed 1 m above the Mean Low Water Mark (MLW) at the sites. Indigenous mussels from each site (the locals) were also placed in baskets and treated similarly to the transplants.

Initially, mussels were transplanted from Bantry Bay to Douglas Estuary, referred to as Bantry/Douglas Transplants and from Douglas Estuary to Bantry Bay, referred to as Douglas/Bantry Transplants. These mussels were left *in situ* for 44 days. This period was considered to be more than sufficient for the mussels to acclimatize to their new environment (Bayne *et al.* 1976).

Mussels were also transplanted from Youghal Estuary to Douglas Estuary, referred to as Youghal/Douglas Transplants, and from Douglas Estuary to Youghal Estuary, referred to as Douglas/Youghal Transplants for 72 days. In the case of the Colligan Estuary, mussels were collected from four different sites within the Colligan Estuary (sites 1–4, see Figure 1). Two of these sites (1,2) were above the tannery outflow point while two (3,4) were below. The pollution gradient was defined by digestive gland lipofuscin density and total tissue chromium concentrations (a primary contaminant in the effluent) (Walsh and O'Halloran 1996). Tissue TCMTB concentrations were not measured. Mussels were also transplanted from Youghal Estuary to site 3 in the Colligan Estuary, referred to as Youghal/Colligan Transplants. The reciprocal transplantation was not conducted.

Sample collection

After the respective exposure periods, the mussels were collected from the sites and transported directly to the laboratory in clean plastic containers. Gill and digestive gland tissues were dissected within 24 h of collection and immediately frozen in liquid nitrogen. The tissues were subsequently stored at –70°C until required.

Preparation of cytosol

Typically 4g of each tissue (pooled from a number of individuals, the number required depending on the tissue being studied, i.e. digestive gland : 8–12; gill: 24–32 individuals) was used for preparation of cytosol for GST enzymatic assays or further purification. Tissue homogenization was performed at 4°C using 3–4 volumes of homogenization buffer [10 mM sodium phosphate (pH 7.2), 0.5 M sucrose, 0.15 M KCl, 1.00 mM EDTA and 1.00 mM dithiothreitol (DTT)] with a polytron homogenizer (3000 rpm × 30 s). The homogenate was subsequently centrifuged for 1 h at 4°C, 23000 *g*, using a Sorvall Superspeed RC2-B automatic refrigerated centrifuge. The resulting supernatant (cytosol) was retained for further purification.

Affinity purification procedure

Column chromatography was carried out at 4°C. Cytosol was filtered through glass wool and the filtrate rapidly desalted using a Sephadex G-25 column (17 × 3.0 cm) pre-equilibrated in 10 mM sodium phosphate buffer (pH 7.2). The resulting eluate, with conductivity equal to that of the buffer, was passed through a GSH–agarose affinity (5 × 1 cm) column pre-equilibrated with the same buffer.

Non-specifically bound material was eluted by passing five column volumes of 10 mM sodium phosphate buffer (pH 7.2) containing 200 mM NaCl through the column. The specifically-bound material was removed using 10 mM Tris-Cl buffer (pH 9.0) containing 200 mM NaCl and 30 mM GSH. All the specifically-bound material typically eluted in 15–20 ml, and this was subsequently passed through a second G-25 column (16 × 3.0 cm) pre-equilibrated with 10 mM Tris-Cl buffer (pH 8.5) to remove the GSH and salt. The eluate was collected in 5 ml fractions. The fractions displaying GST activity (using CDNB as substrate) were pooled and stored at –70°C in 10–15 ml fractions until required for further chromatography.

Fast protein liquid chromatography (FPLC)

FPLC has previously been widely used in purification of GSTs from mammalian sources (Ålin *et al.* 1985, Meyer *et al.* 1991). Ten ml (3–5 mg) desalted, affinity-purified extract was applied to the FPLC (Pharmacia). Separation was achieved using Mono Q (HR 5/5) anion exchange chromatography at room temperature. Protein was detected by measuring absorbance at 280 nm using a single path, dual beam flow-through UV-1 monitor (Pharmacia). The buffers used were as follows: buffer A, 10 mM Tris-Cl (pH 8.5), buffer B: 10 mM Tris-Cl (pH 8.5) containing 1.0 M NaCl. A gradient of 0 to 40% B over 17 min was used to achieve separation of the isoenzymes. As the protein peaks eluted from FPLC they were collected and immediately placed on ice.

Measurement of GST enzymatic activity

GST catalytic activity using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate was determined using the method of Habig *et al.* (1974). The buffer used was 0.1 M sodium phosphate. The GSH required for these assays was dissolved in 0.1 M disodium hydrogen phosphate solution. The CDNB was dissolved in ethanol. In all cases the final concentration of ethanol in the assay mixture was 5% (v/v).

Determination of intracellular GSH concentration

GSH concentrations were determined by the method of Griffith (1980) in which GSH is sequentially oxidized by 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB] and reduced by NADPH in the presence of GSH reductase (EC 1.6.4.2). The rate of 2-nitro-5-thiobenzoic acid formation was monitored at 412 nm and the GSH concentration was determined utilizing a standard curve. Both reduced (GSH) and oxidized (GSSG) GSH are detected by this method. Solutions of 3 mM NADPH (solution I) and 6mM DTNB (solution II) were prepared in 125 mM sodium phosphate buffer (pH 7.5) containing 6.3mM EDTA. GSH reductase solution containing 50 units per ml was made up in water. The assay was performed at 30°C.

Estimation of dry/wet weight ratios

A sub-sample of the pooled tissue used for biochemical analysis was taken from the respective sites and its wet weight determined. The tissues were subsequently dried at 70°C to a constant weight using a Gallenkamp OV-160 oven. The tissues were then re-weighed and the dry/wet weight ratio calculated. This dry/wet weight ratio was determined at least in triplicate for each tissue sample from the respective sites.

The dry/wet weight ratios were determined for all tissue samples. These ratios were used to calculate the GST specific activity g^{-1} dry weight.

Statistical analysis

Comparison of mean values where appropriate were analysed by Student's *t*-test or one-way analysis of variance using the minitab statistical package (Ryan *et al.* 1985).

Results

Influence of site pollution status on GST specific activity

The results for differences in GST specific activities between sites are summarized in Table 1. The mean digestive gland GST specific activity was found to be similar between Douglas (locals 1) and Bantry (locals). By comparison, specific activity in the gill was higher in Douglas (local) samples relative to Bantry (locals) ($p<0.05$). Transplantation from Bantry to Douglas also resulted in a significant increase in gill specific activity samples ($p<0.01$). Conversely, when mussels were transplanted from Douglas to Bantry, i.e Douglas/Bantry Transplants, the GST specific activity in the gill was significantly reduced relative to the Douglas locals and approached a value similar to that of the Bantry locals ($p<0.05$). Unfortunately, as insufficient tissue was available to determine the wet/dry weight tissue ratio of digestive gland from the Bantry Transplant animals, the changes in GST specific activity could not be assessed for digestive gland in this group.

In the subsequent comparison between Douglas estuary and Youghal estuary, a similar pattern was evident, i.e. increased enzyme activities were recorded in Douglas gill tissue in both locals ($p<0.001$) and transplants ($p<0.001$) relative to Youghal mussels. This pattern was mirrored in the digestive gland (Table 1). In addition, transplantation of mussels from the Douglas estuary to Youghal led to a net decrease in enzyme activity in both tissues, i.e. gill ($p<0.001$); digestive gland ($p<0.001$).

These latter samples were also analysed for total intracellular GSH concentration (Table 2). However, no apparent pattern in intracellular GSH concentrations was observed.

	Digestive gland		Gill	
Site	IU g^{-1} dry wt Mean ± s.e.	Dry/wet wt Conversion ratio	IU g^{-1} dry wt Mean ± s.e.	Dry/wet wt Conversion ratio
Bantry locals	6.29 ± 0.14	3.75	15.62 ± 0.43	9.07
Bantry/Douglas Transplants	ND	ND	21.18 ± 0.30**	11.73
Douglas locals 1	6.46 ± 0.37	4.62	18.22 ± 0.85*	11.39
Douglas/Bantry Transplants	ND	ND	14.37 ± 0.46*	9.43
Youghal locals	7.15 ± 0.35	4.26	9.46 ± 0.40	9.19
Youghal/Douglas Transplants	10.42 ± 0.26***	5.04	22.75 ± 0.90***	11.00
Douglas locals 2	9.16 ± 0.28***	5.16	22.89 ± 1.16***	10.15
Douglas/Youghal Transplants	7.21 ± 0.38***	4.31	12.78 ± 0.02***	8.48

Table 1. GST specific activity of cytosolic extracts (IU g^{-1} dry weight) for tissues of *M.edulis* locals and transplants from Douglas, Youghal and Bantry sites.

The results are expressed as mean values ± s.e. ($n = 4$). Douglas locals 1 refer to samples taken during the Douglas/Bantry comparison and Douglas locals 2 to those sampled during the Douglas/Youghal comparison. Factors to convert values, by division, to IU g^{-1} wet weight are also given. ND = Not determined; IU = International Unit; transplants are given as source/destination. Significance values: *$p<0.05$; **$p<0.01$; ***$p<0.001$ refer to changes between source and corresponding transplant or between corresponding sources, e.g. Douglas locals 1/Bantry locals.

Site	GSH concentration (nmoles g^{-1} dry wt. Mean ± s.e.) Gill	Digestive gland
Douglas locals	2039.9 ± 230.9	2595.5 ± 40.7
Douglas/Youghal Transplants	1709.0 ± 130.4	2530.6 ± 153.2
Youghal locals	1912.4 ± 227.6	2914.8 ± 138.5*
Youghal/Douglas Transplants	2979.4 ± 477.9**	3981.8 ± 219.2*

Table 2. GSH concentrations (nmoles g^{-1} dry weight) for tissues of *M. edulis* from the sites used in the transplantation experiments.

Site descriptions as in Table 1. The results are given as the mean values ± s.e. ($n = 4$). Significance values: *$p<0.05$; **$p<0.01$ refer to changes between source and corresponding transplant or between corresponding sources, i.e. Douglas/Youghal.

FPLC analysis of gill and digestive gland affinity-purified extracts from M. edulis

The GSTs from the gill and digestive gland of *M. edulis* have previously been purified and biochemically characterized (Fitzpatrick and Sheehan 1993, Fitzpatrick *et al.* 1995b). FPLC analysis of GSH agarose affinity-purified extracts from these tissues reveals the presence of four protein peaks, 1–4 (Fitzpatrick and Sheehan 1993). The mean ± s.e. values for the peak areas from samples taken from Douglas and Youghal locals and the reciprocal transplants between these sites are summarized in Table 3. No significant difference between the relative levels of the GST-like proteins was observed.

Effects of oxidative stress on GST specific activity

The results of the leather tannery effluent exposure study are summarized in Table 4. Higher GST specific activity was observed in the gill samples from Youghal mussels than in any of the four sites from the Colligan Estuary ($p < 0.01$). Conversely in the digestive gland samples, lower activity was observed in Youghal samples compared with samples from the Colligan Estuary ($p < 0.01$). In particular, no pattern in the GST specific activity was observed in either gill or digestive gland tissue in relation to the pollution or salinity gradient within the contaminated estuary. Furthermore, when mussels were transplanted from Youghal to site 3 in the Colligan estuary (nearest to the effluent outfall), no increase in GST activity was detectable in either tissue.

Discussion

Increases in GST specific activity have been documented in laboratory exposures of bivalves to various organochemicals (Livingstone 1991) such as dieldrin and lindane (Boryslawskyj *et al.* 1988). In contrast, there is no evidence to suggest that exposure of mussels to metals (e.g. Cu, Mn, Fe, and Pb) results in elevated GST levels (Regoli and Principato 1995). Therefore, the potential exists for their use as a possible specific index of organic chemical pollution in the environment.

Oxidative stress is believed to result in elevated levels of GST in mammalian cell lines (Daniel 1993). To investigate this possibility in *M. edulis* we analysed mussels exposed to tannery effluent containing the probable aromatic pro-oxidant TCMTB (Walsh and O'Halloran 1996). As no pattern of induction was apparent in exposed animals, this leads us to conclude that oxidative stress *per se* does not appear to induce GST specific activity in *M. edulis*. In addition, it is apparent that the elevation in activity observed in mussels from Douglas Estuary (Table 1) is similarly not a result of oxidative stress as no evidence of increased peroxidation was detected in the tissues of these animals, as indicated by the low incidence of lipofuscin in the digestive cells of these animals (Walsh and Fitzpatrick, unpublished observations).

Therefore, as regards the cause of this elevated GST specific activity observed in samples from Douglas Estuary, it might be suggested that salinity stress may play a role. However, as the results of the Colligan Estuary study show, no change in GST activity was evident in mussels sampled from their upper limit of distribution (Colligan 1) in the estuary to an almost fully marine site (Colligan 4). This therefore suggests that salinity variation is an unlikely influence. Alternatively, the increase observed may be a true reflection of exposure to a chemical or chemicals in the estuary which have previously been shown to specifically induce GST activity in invertebrates, e.g. lindane, dieldrin (Boryslawskyj *et al.* 1988), PAHs and PCBs (Lee 1988).

	Site:	Douglas Locals (2)		Douglas Transplants (2)		Youghal Locals		Youghal Transplants	
	Tissue:	Digestive gland	Gill	Digestive gland	Gill	Digestive gland	Gill	Digestive gland	Gill
Peak	1	5.1 ± 1.0	4.1 ± 3.2	7.9 ± 2.2	2.6 ± 0.2	7.1 ± 1.1	2.2 ± 0.1	7.1 ± 2.2	2.0 ± 0.2
Peak	2	70.4 ± 4.9	64.0 ± 2.8	63.6 ± 2.4	72.4 ± 3.3	66.9 ± 0.8	60.8 ± 3.8	66.6 ± 7.6	62.2 ± 5.7
Peak	3	16.5 ± 3.3	24.6 ± 0.1	20.4 ± 1.8	16.4 ± 1.9	17.6 ± 1.1	27.3 ± 4.6	15.1 ± 1.7	24.8 ± 4.6
Peak	4	7.9 ± 1.6	7.2 ± 0.5	8.0 ± 0.7	8.6 ± 1.3	8.4 ± 0.9	9.7 ± 1.0	11.2 ± 5.9	11.0 ± 1.4

Table 3. The relative amount (%) of each GST-like protein present in the affinity-purified extracts of *M. edulis* digestive gland and gill tissue from the respective sites (mean ± s.e.).

The total area of the four peaks was taken as 100%. The individual peak areas were measured by the FPLC programme controller ($n = 3$). Site descriptions as in Table 1.

Site	Gill		Digestive gland	
	IU g^{-1} dry wt (mean ± s.e.)	Dry/wet wt conversion ratio	IU g^{-1} dry wt (mean ± s.e.)	Dry/wet wt conversion ratio
Youghal locals	21.68 + 1.11	12.42	5.64 ± 0.18	4.05
Youghal/Colligan Transplants	15.92 ± 0.22**	10.02	5.76 ± 0.28	4.08
Colligan 1	19.15 ± 1.52	9.91	6.58 ± 0.10**	3.98
Colligan 2	17.92 ± 0.64**	9.57	7.16 ± 0.22**	4.24
Colligan 3	15.73 ± 0.79**	9.29	6.36 ± 0.07**	4.17
Colligan 4	16.23 ± 0.57**	9.10	6.50 ± 0.23**	4.26

Table 4. GST specific activity (IU g^{-1} dry weight; mean ± s.e.; $n = 4$) of gill and digestive gland tissues of *M.edulis* sampled from tannery contaminated sites in the Colligan Estuary and from the Youghal Estuary reference site.

Colligan sites 1–4 refer to a gradient along the Colligan Estuary (see Figure 1). Factors to convert values, by division, to IU g^{-1} wet weight are also given. Transplants are given as source/destination. Differences between mean values: **$p<0.01$ refer to differences relative to Youghal reference values.

In this context, elevated concentrations of organochlorines and PCBs have been reported in tissues of mussels taken from Cork Harbour compared with other sites around the Irish coast (Dineen 1989). Alternatively, the active ingredient in some antifouling paints, i.e. tri-butyltin (TBT), may also be responsible. Douglas Estuary is located adjacent to the main commercial shipping channel in Cork Harbour and thereby probably has increased levels of TBT due to leaching of this compound from such paints. Studies on the morphological defects in mussels and oysters in and around the Douglas Estuary indicate elevated levels of this compound are present here (D. Manchin, pers. comm.). Moreover, as GSTs have been implicated in the detoxification metabolism of this compound in many marine animals including mussels (Lee 1991), the elevated GST activity observed could be a result of organotin exposure. However, although the evidence for the presence of a GST inducer is good, the identification of the causative agent requires further clarification.

Compared with the evidence for variation in GST specific activity between sites, there appears to be no specific effect on the relative amount of the individual GST-like proteins based on FPLC analysis (Table 2). Of the four GST-like proteins isolated from *M. edulis* tissues, only peak 1 (GST 1) displays detectable catalytic activity with any of the commonly used GST substrates (Fitzpatrick *et al.* 1995b). However, as the specific activity varies significantly for both gill and digestive gland samples, particularly for the Douglas/Youghal and reciprocal transplants (Table 1), it was surprising that no change in enzyme levels (particularly for peak 1) was observed. This may be due to the fact that relative levels were measured, hence if all the GST-like proteins were induced to the same extent no differences in these levels would have been detected. Uptake of xenobiotics by mussels has been well documented. However, the xenobiotics are metabolized at a very slow rate compared with that in mammals (Livingstone 1991). Possible reasons for this could be very low catalytic efficiency of the detoxification enzymes in these organisms or that they rely more on binding proteins to sequester xenobiotics as a mechanism of detoxification. Despite having no catalytic activity, the protein corresponding to peak 2 (GSH-binding protein), accounts for approximately 70% of the protein from GST affinity-purified extracts from several different *M. edulis* tissues (Fitzpatrick 1994). In addition, recent work has shown that this protein binds several lipophilic compounds (A. Power, pers. comm.). It is possible therefore, that this protein may be involved in non-catalytic binding of various xenobiotics which enter the cell and thereby prevent interaction with DNA and other proteins (Ketterer *et al.* 1967, Smith *et al.* 1977). The various GST-like proteins may have different binding properties for many different types of chemicals and as such help protect the cell from this threat. In view of this, it may be more appropriate to investigate changes in the actual levels of proteins such as the GSH-binding protein as a more sensitive index of exposure of these organisms to chemical pollution. Therefore, it would be interesting to measure the actual levels of the individual proteins for example via HPLC (Pascual *et al.* 1991). In order to carry out such a study it is imperative that an equivalent amount of sample be analysed in each case. This is not easy given the variation in wet weight and protein content (as measured by the Lowry assay procedure), from site to site (Fitzpatrick, unpublished observations). However, by using DNA content as an index of cell number, this problem could possibly be overcome.

In conclusion the evidence provided indicates that some factor in the industrialized Douglas Estuary results in elevated GST specific activity in mussel gill and digestive gland tissues. The results from the tannery study suggest that salinity and oxidative stress, in particular, are not factors in this induction. Further work is therefore prompted in order to verify whether the induction in Douglas Estuary is specific to a xenobiotic at the site. In addition, the FPLC study suggests that quantitative measurement of the GST concentrations present may also be useful as indices of the induction of non-catalytic GST proteins.

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