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Glutathione *S*-Transferase Activity in Mussels, *Mytilus edulis*, Exposed to Discharges from an Aluminum Smelter

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Loch Leven is a sea loch situated on the West Coast of Scotland (Fig. 1). At the head of the loch is an aluminium smelter which discharged an aqueous effluent containing polycyclic aromatic hydrocarbons (PAHs) until closure in June 2000. Concern had been expressed over the possible effects of the discharge on marine shellfish (McIntosh *et al.*, 2001a). This study addressed the effects of the discharge on the common mussel, *Mytilus edulis*.

Mussels have been used previously to monitor the environment surrounding aluminium smelters, mainly as bioaccumulation sentinels (Näf *et al.*, 1994; Baumard *et al.*, 1999). However, difficulties have been encountered in demonstrating biological effects of PAHs in the discharges from smelters. Some studies have shown little or no biological impacts of sediments containing high concentrations of PAHs (Paine *et al.*, 1996; Næs *et al.*, 1999) and it has been suggested that the bioavailability of the PAHs from smelters is low. Knutzen (1995) suggested that biological effects caused by PAHs from aluminium smelters will only occur at extremely high PAH concentrations; these concentrations were considerably beyond the toxicity thresholds for the species calculated in laboratory studies (Knutzen, 1995). High variances in some biological effect measurements contribute to the difficulty in demonstrating effects (Thomas *et al.*, 1999). In this paper, data from an on-going study into various ecotoxicological aspects of the aluminium smelter effluent in Loch Leven are presented. The biological effect measurements, glutathione *S*-transferase activity and neutral red retention time, were made on mussels from various locations in and around Loch Leven.

Glutathione *S*-transferase (GST) activity is a measure of the potential for mussels to metabolise PAHs by conjugation to glutathione. GST activity in mussels has been investigated as a potential biomarker of contamination (Kaaya *et al.*, 1999) and has been shown to be elevated in scallops close to an aluminium smelter in Norway (Næs *et al.*, 1999). Elevated GST activity has been shown in mussels (*Modiolus* and *Bathymodiolus*, gill and hepatopancreas) associated with PAH contamination near petroleum seeps (Willet *et al.*, 2000). However, previous laboratory studies on mussels have shown that the activity of this enzyme in gill tissue can be inhibited by high PAH doses (Akcha *et al.*, 2000). Thus, GST activity may be induced in

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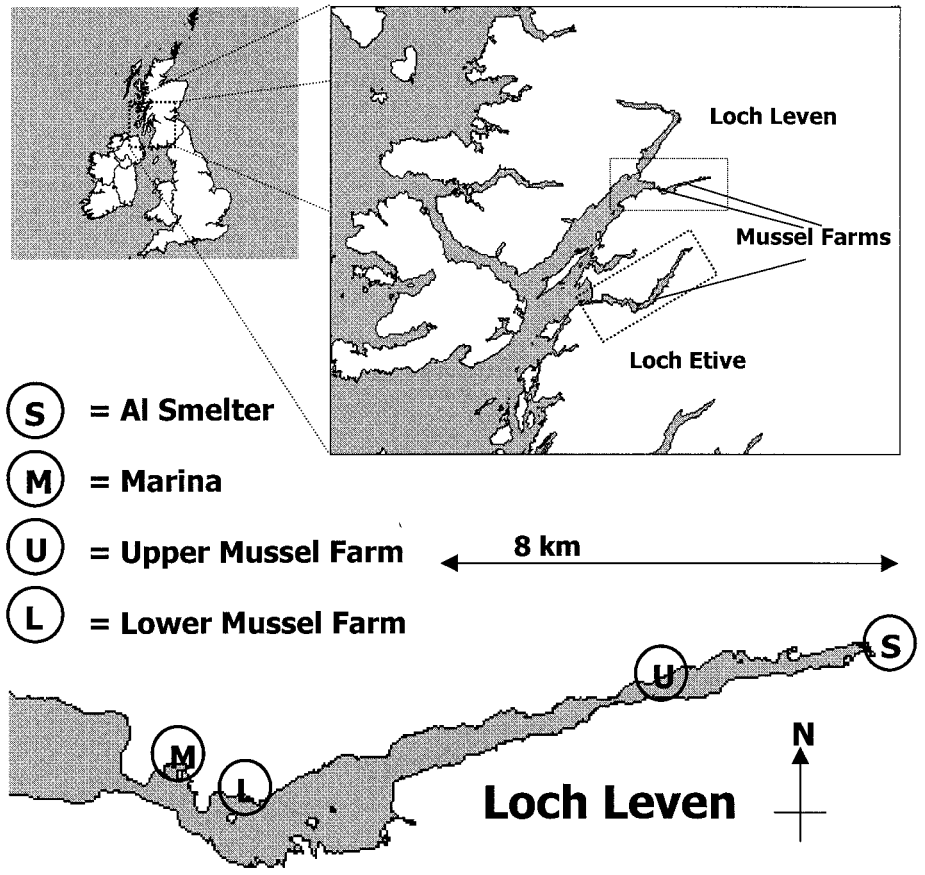

Figure 1. Loch Leven and Loch Etive in western Scotland.

instances of high PAH contamination, but may be reduced at extreme levels of exposure and signs of deleterious higher-order effects may become apparent.

Neutral red retention (NRR) time is a measure of lysosomal stability in haemocytes and has been used as an index of the stress in mussels. Sensitivity of this response to PAH exposure has been demonstrated in the laboratory and the field (Lowe and Pipe, 1994; Moore *et al.*, 1996; Grundy *et al.*, 1996; Krishnakumar *et al.*, 1994).

In this study, GST and NRR are compared in farmed and wild mussels from Loch Leven, and Loch Etive, a similar loch well known to have low PAH input. Mussels were also transplanted between the two lochs to support evidence of the biological effects.

MATERIALS AND METHODS

Mussels were collected from wild (May 2000) and farmed (April 2000) sites. Five wild mussel sites along the north side of Loch Leven were labelled A to E, from west to east (Fig. 2). These animals were dissected and the hepatopancreas excised and snap frozen in liquid nitrogen immediately after collection.

The farmed sites were in the upper and lower parts of Loch Leven, and also in Loch Etive. Batches of mussels were exchanged between Loch Etive and Loch Leven. Farmed mussels were collected from:

Upper Leven Mussel Farm (Fig. 1 – inset and main picture)
Lower Leven Mussel Farm (Fig. 1 – inset and main picture)
Etive Mussel Farm (Fig.1 – inset only)
Etive Mussels transplanted (Sept. 1999) to the Upper Leven Mussel Farm
Etive Mussels transplanted (Sept. 1999) to the Lower Leven Mussel Farm
Upper Leven Mussels transplanted to Etive Mussel Farm (March 2000)

Mussels were put in damp insulated boxes and brought to FRS Marine Laboratory, Aberdeen. Mussels were either dissected for GST measurement (as above) or put into holding tanks for NRR measurements.

To measure GST activity, each individually excised mussel hepatopancreas was homogenised with 2 mL of homogenising buffer containing 25.48 g/L of NaCl, 13.06 g/L of $MgSO_4$, 0.75 g/L of KCl, 1.47 g/L of $CaCl_2$, 4.77 g/L Hepes, 0.372 g/L of EDTA and 0.154 g/L of DTT. The homogenate was then centrifuged at 10 000 g for 20 minutes and the S9 fraction removed for analysis. These procedures were conducted in chilled conditions. The GST activity of the fraction was then quantified on a Varian Carey 3E spectophotometer by conjugation of glutathione to 1-chloro-2,4-dinitrobenzene (Habig and Jakoby, 1981). The activity was normalised to the protein content of the sample, which was determined by a Biorad DC protein assay kit using established methodology (Lowry *et al.*, 1951) and quantified on an Argus 300 plate reader. Statistical analyses for GST measurements were conducted by first testing for normality using Ryan-Joiner tests, then using ANOVA for analysing statistical differences between all groups. Finally, Tukey's pair-wise comparisons were used to detect where significant differences lay.

For the neutral red retention assays, mussels were brought from the study sites and held in filtered seawater tanks for one or two days before being subjected to a NRR assay. This period was short enough to avoid recovery from PAH stress (Lowe, 1995). The assay was conducted according to the standard operating procedure set out in the European Biological Effects Quality Assurance in Marine Monitoring (BEQUALM) project (based on Lowe and Pipe, 1994). Statistical analyses for NRR measurements were conducted by using a non-parametric Kruskal-Wallis test.

All assays were conducted at FRS Marine Laboratory, which operates a quality system accredited by the UK Accreditation Service.

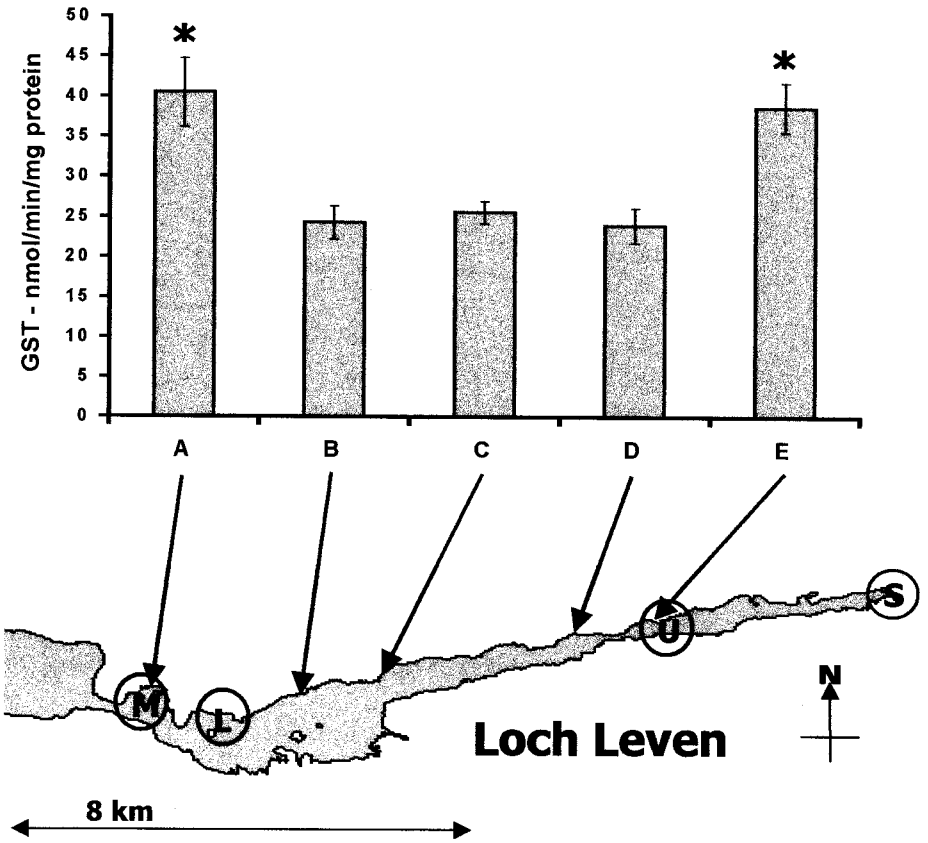

Figure 2. Mean glutathione *S*-transferase activity in hepatopancreas from wild mussel populations along the north side of Loch Leven. Error bars indicate standard error of the mean (n=15 at each site). The two sites marked (*) are significantly different from the rest of the sites, but not from each other (ANOVA, $P<0.001$). Site A is a marina, and site E is close to the upper mussel farm.

RESULTS AND DISCUSSION

Individual GST activities in the wild mussel hepatopancreas ranged from 8 to 85 nmol/min/mg protein (Fig. 2) and were tested for normality (Ryan-Joiner). All groups were normally distributed. ANOVA demonstrated that sites A (Marina) and E (Upper Leven) had significantly higher activity than sites B, C, or D ($F=8.05$, $P<0.001$, followed by Tukey). There was no significant difference between sites A and E.

Individual GST activity, ranging from 0.3 to 90 nmol/min/mg protein, was normally distributed within the normal and transplanted farmed mussel groups

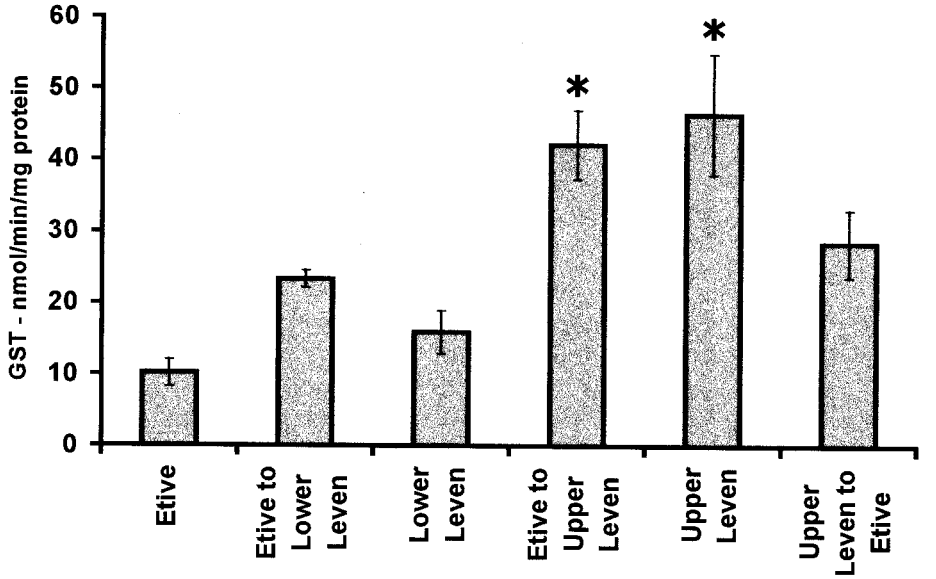

Figure 3. Mean glutathione *S*-transferase activity in mussel hepatopancreas from farmed and transplanted mussels in Loch Leven and in Loch Etive. Error bars indicate standard error of the mean (n=10 at each site). Mussels cultured at the upper Leven mussel farm and mussels transplanted from Loch Etive farm are significantly different from all others but not from each other (ANOVA, $P<0.001$; marked with *).

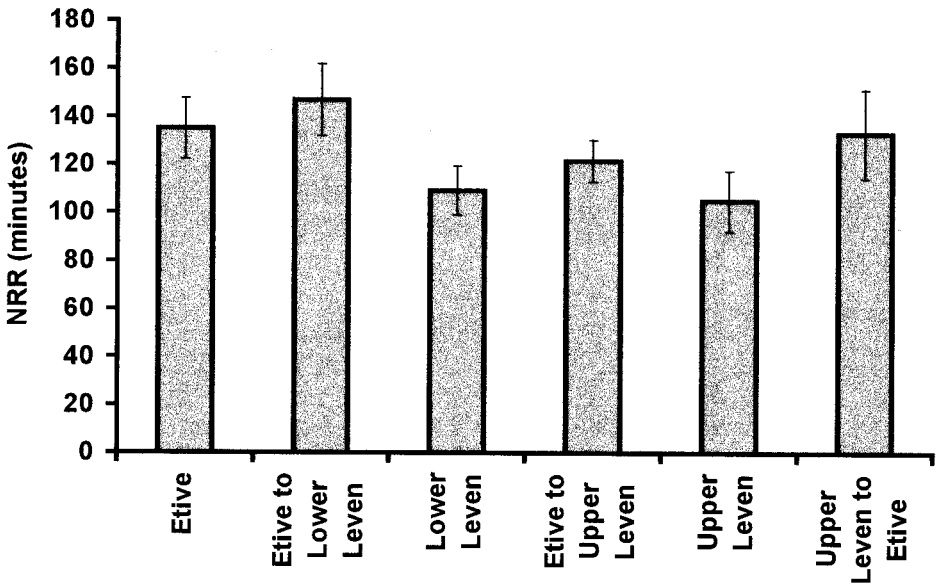

Figure 4. Mean neutral red retention times of haemocytes from farmed and transplanted mussels from Loch Leven and Loch Etive. Error bars indicate standard error of the mean (n=10 at each site). No significant differences ($P<0.05$) were found between sites (Kruskal-Wallis, $P=0.1$).

(Ryan-Joiner). ANOVA showed that GST activity (Fig. 3) was significantly different between groups ($F=10.48$, $P<0.001$). Mussels from the upper farm in Loch Leven (normal and transplanted) were not significantly different from each other. Likewise, the remaining four groups were not significantly different from each other. However, these two sets of groups were significantly different from each other using Tukey's test.

Neutral red retention assays of the different groups of farmed mussels (Fig. 4) showed no significant differences between groups ($P=0.1$, Kruskal-Wallis).

No wild mussels were found on the shore closer to the smelter than the sparse population found at site E (3 kilometres from the discharge). This is likely to be the natural upper limit of mussels in the loch, most probably due to the low salinity in surface waters. It is considered unlikely that this limit is an effect of the smelter, since the mussels at the adjacent farm were growing well. These farmed mussels were suspended below the surface layer in the water column, to avoid the low salinity water (*G. Salvarli, pers. comm.*). The increased GST activity in mussels found at site E and at the upper Leven mussel farm is thought to be due to the smelter effluent containing PAHs. The wild and farmed sites are within 100 metres of each other.

Mussels from the Loch Etive mussel farm and the lower Leven mussel farm showed low GST activity compared to those at the upper Leven farm, suggesting that there was insufficient exposure to PAHs at the lower Leven farm to raise GST activity. The high GST activity in wild mussels at site A is thought to be caused by contaminants introduced within the marina. A previous study in this area has shown signs of petrogenic input in the lower loch (McIntosh *et al.*, 2001b), possibly from boat activity in and around the marina.

The lack of significant differences in the neutral red retention data suggest that, despite the high GST activity of mussels at Upper Leven, those mussels are not significantly stressed by the PAHs contained in the smelter effluent. This is supported by the observation that mussels are growing well at the upper mussel farm *(G. Salvarli, pers. comm.*).

Previous field studies on GST in mussels associated with PAH contamination have shown elevated activity at high PAH concentrations (Willet *et al.*, 2000). However, GST activity was depressed at extreme concentrations of PAH in the laboratory, (Akcha *et al.*, 2000). Therefore, it appears that the PAHs in upper Loch Leven were not sufficiently bioavailable to depress GST activity or NRR, but sufficient to induce GST activity. This implies that, although upper Leven mussels must be devoting energy to deal with the PAHs, it is not to the extent that there are noticeable health effects.

In conclusion, mussels in the upper part of Loch Leven show significantly elevated levels of GST activity, whereas mussels found in the lower part of the loch did not. The elevation is most likely due to PAHs in the smelter discharge. The increase in

GST activity suggests the mussels are metabolising the PAHs, and the neutral red retention data demonstrate that the mussels remained healthy. Measurements of stress effects, such as NRR, along with GST activity could help to ensure that the GST data are correctly interpreted in terms of exposure level, and also potentially provide links with higher order effects.

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