THE APPLICATION OF THE ERYTHROCYTE MICRONUCLEUS ASSAY AS A POTENTIAL BIOMARKER OF CONTAMINANT EXPOSURE IN THE EUROPEAN FLOUNDER (*Platichthys flesus*): RESULTS FROM LABORATORY AND FIELD STUDIES

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Introduction

The erythrocyte micronucleus assay (MN) has previously been used with varying success as a cheap and rapid *in vivo* assay for detecting genotoxic damage in a number of fish species. The European flounder (*Platichthys flesus*) is a marine flatfish common to UK coastal waters and has served as a model species for investigating contaminant related effects in estuarine ecosystems (Stentiford *et al.*, 2003) (Figure 1). Furthermore, flounder have recently been identified as a sentinel species under the UK National Marine Monitoring Programme (NMMP). The current study was initiated to investigate the suitability of the erythrocyte MN assay to be used as a biomarker test for determining genotoxic exposure in European flounder.

Figure 1: The European flounder (Platichthys flesus). Photo courtesy of B. Picton (http://www.habitas.org.uk/marinelife/)

Materials and Methods

Tank studies

Two studies were conducted exposing fish daily to a diet spiked with four PAHs (phenanthrene, pyrene, benzo(a)pyrene and benzanthracene). One study simulated the high levels of PAH that may be ingested following an oil spill and was run for 4-months and one reflected the concentrations of PAH present in prey items at moderately contaminated sites (Baumard *et al.*, 1998) and was run for 6-months. The nominal dosing ranges of total PAHs were 100 and 500 mg kg^{-1} (food weight) for the 4-month and 0.1, 5 and 50 mg kg^{-1} for the 6-month experiment. Control groups consisted of fish receiving the feed spiked with the solvent carrier (hexane) only. Preparation of the spiked feed and husbandry conditions are described in detail elsewhere (Reynolds *et al.*, *in press*). Blood smears were collected, stained with giemsa and scored for the presence of MN according to the criteria of (Al-Sabti and Metcalfe, 1995).

Field sampling

Flounder were collected from a series of UK estuaries (Figure 2) known to be impacted by contaminants (Law *et al.*, 1997; Stentiford *et al.*, 2003). Additional fish were sampled from a reference site, River Alde, deemed to be relatively uncontaminated.

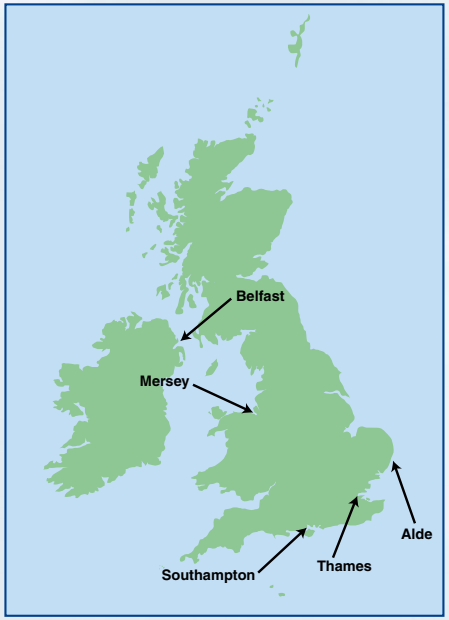

Figure 2: Estuarine sample locations around the UK

Results

Laboratory experiments

Flounder exposed to a diet spiked with 500 mg kg^{-1} PAH for 4 months resulted in a significant induction ($p<0.05$) of MN in erythrocytes relative to the fish fed food spiked only with the solvent carrier (Figure 3).

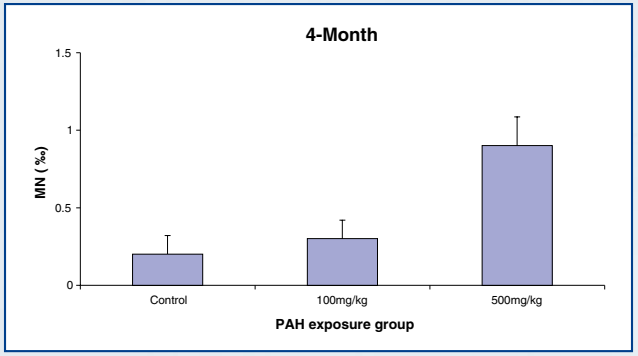

Figure 3: 4 month tank exposure experiment, mean ± SE (n=5)

In the 6 month exposure experiment no statistical differences ($p<0.05$) in erythrocyte MN frequency were detected between the treatment groups (Figure 4).

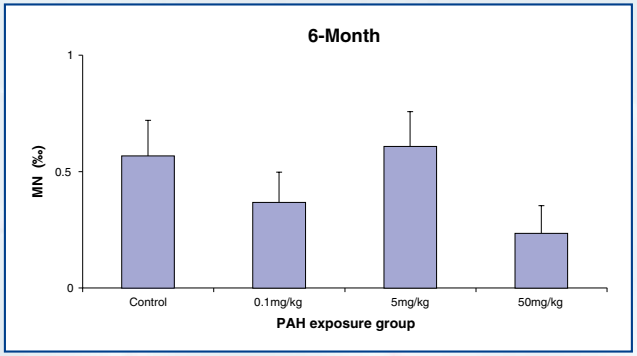

Figure 4: 6 month tank exposure experiment, mean ± SE (n=13-15)

Field sampling

The frequencies of MN in flounder erythrocytes are displayed in Table 1. No significant differences ($p<0.05$) could be detected between the estuaries sampled.

Table 1: Erythrocyte MN frequencies in flounder from UK estuaries

Sample location	Number of fish sampled	Mean ± SE
Southampton	11	0.41 ± 0.16
Mersey	9	0.17 ± 0.08
Belfast	7	0.43 ± 0.17
Thames	15	0.66 ± 0.3
Alde	15	0.27 ±0.18

Conclusions

- These preliminary experiments suggest that the erythrocyte MN assay as applied to European flounder is not of the required sensitivity to be used as a routine biomarker of chronic contaminant exposure within UK estuaries.
- Experiments are continuing to investigate whether staining erythrocytes with acridine orange, which is able to distinguish mature and immature erythrocytes, can increase the sensitivity the flounder erythrocyte MN assay.

Acknowledgement

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References

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