

Introduction

A diverse array of potentially harmful contaminants enters the estuarine and coastal waters of the UK. Conventional bioassay methods and chemical analysis techniques have allowed scientists to assess the concentrations of specific contaminants in water and sediment, and the potential biological effects of these on parameters such as survival, growth and reproduction. However, the need to detect and assess the effects of contamination at ever lower concentrations and in more complex mixtures have led to the development of a range of sub-lethal indicators of exposure to, and effects of, contaminants and other environmental stressors. These indicators are collectively referred to as 'biomarkers' and can be either specific (monitoring the presence/effects of specific chemical classes) or general. This study describes the use of two specific enzyme biomarkers to monitor the exposure and effects of specific contaminants in estuarine and coastal fish populations. The studies are covered in more detail in Kirby *et al.*, (1999b) and Kirby *et al.*, (2000).

Sampling and analysis

- Flounder (*Platichthys flesus*) were captured using 2-3 metre beam trawls deployed from research vessels in the period from September to December 1997.
- Trawls were towed at 3-4 knots for 20-30 minutes to minimise stress to the fish.
- Fish were kept alive and dissections were carried out within one hour of capture.
- The livers were excised, weighed, and immediately stored in liquid nitrogen for **Ethoxyresorufin-O-deethylase (EROD)** analysis.
- A strip of muscle taken from the dorsal surface near to the spine was immediately stored in liquid nitrogen for **Cholinesterase (ChE)** analysis.
- Condition factor (**CF**), Hepato-somatic index (**HSI**) and Gonado-somatic index (**GSI**) were calculated from whole fish and tissue weights.
- Liver tissue was analysed for **EROD** activity using a modification of the method described by Stagg and McIntosh (1998).
- Muscle tissue was analysed for **ChE** activity using a modification of the technique described by Bocquené and Galgani (1998).
- **EROD** and the **ChE** activities were normalised to protein content, measured via the method described by Bradford M.M. (1976).
- Bulk liver samples from some estuaries were analysed for **PAHs** and **PCBs** (see table 1).
- Chemical analysis of estuarine water from representative sites was also undertaken for a range of **Organophosphate** and **Carbamate** pesticides (see table 2).

Table 1 Mean total PAH and PCB in bulked samples of Flounder liver.

Location	PAH (µg/kg wet weight)	PCB (µg/kg wet weight)
River Alde	109.7	32.0
River Tees	365.0	N/A
River Humber	71.2 – 100.6	424.0
River Mersey	363.0	1082.0

EROD results

- 8 of the 15 stations sampled showed significantly ($p < 0.05$) elevated EROD activities compared to the Alde reference site (see figure 2).
- Estuaries ranked in order of potential contaminant exposure: - **Mersey>Tees>Wear>Tyne>Humber>Southampton>Alde**.
- Greatest induction was shown at Bromborough site in the Mersey: a maximum of four-fold increase in induction over the reference value.
- Correlation analysis of mean EROD activity with mean GSI, HSI and CF revealed no obvious relationships between the estuaries. However, female mean EROD was found to be strongly negatively correlated to GSI ($r = -0.84$) and CF ($r = -0.80$) from sites sampled in the Tyne.

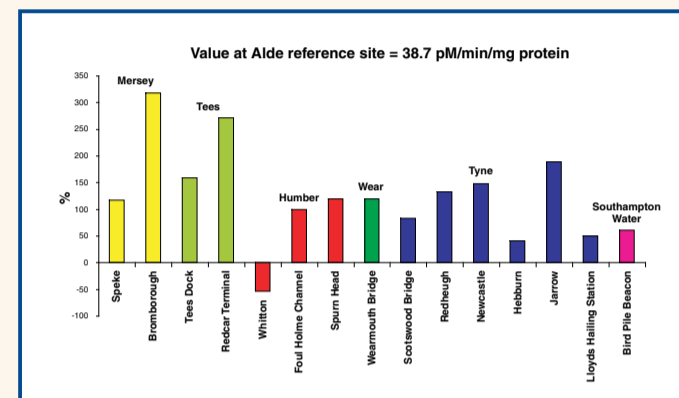


Figure 2 % Increase in EROD Activity compared to the Reference Site.

ChE results

- 12 out of 16 sites showed significantly ($p < 0.05$) lower ChE activities compared to the Alde (see figure 3).
- Estuaries ranked in order of potential neurotoxicity: - **Mersey>Tyne, Tees, Tamar, Humber>Alde>Wear**.
- Results from the Tyne estuary shows a clear decreasing trend downstream.
- Correlation analysis of mean ChE activity with gender, mean GSI, HSI and CF revealed no obvious relationships. Size, however, showed some influence.

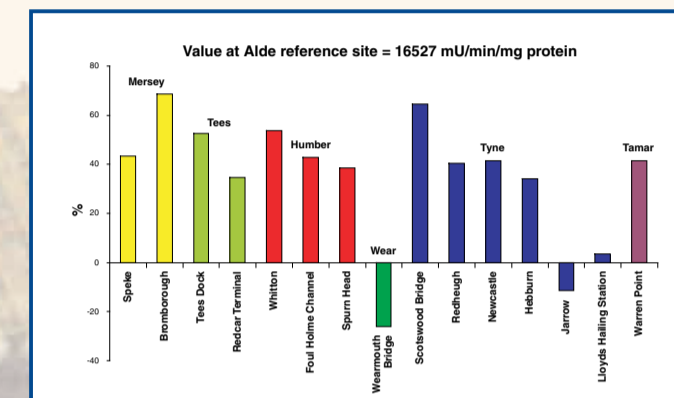


Figure 3 % Inhibition in ChE Activity compared to the Reference Site.



Figure 4 Map of sampling sites.

Table 2 Concentrations (ng/l) of Organophosphates and Carbamates in Estuarine water.

Pesticide	LOQ (ng/l)	Alde	Humber	Tamar	Mersey	Tees	Tyne
Organophosphates							
Heptenophos	30	<30	<30	<30	<30	39	<30
Diazinon	20	<20	39-57	<20	36	<20	28-43
Disulfoton	30	<30	32	<30	<30	<30	<30
Etrimfos	10	<10	<10	26	<10	<10	<10
Fenthion	10	<10	<10	<10	13-29	<10	<10
Chlorfenvinphos	10	<10	25	<10	18-31	<10	31-50
Triazophos	20	<20	48	<20	<20	37	<20
Azinphos-methyl	30	<30	<30	<30	<30	46	<30
Carbamates							
Carbofuran-3-OH	10	<10	<10	<10	<10	26	<10
Carbofuran	2	<2	<2	<2	<2	<2	13-21
Bendiocarb	2	<2	<2	<2	<2	<2	18
Carbaryl	2	<2	<2	<2	<2	18	<2
Ethiofencarb	5	<5	<5	<5	8	<5	32
Pirimicarb	2	<2	<2	<2	<2	<2	12

LOQ = Limit of Quantitation

Conclusions

- This survey shows that the MFO enzyme system in Flounder is significantly induced in a number of English estuaries.
- Estuarine ranking in terms of EROD induction in Flounder closely reflects the hepatic accumulation of MFO inducing compounds in these areas (see table 1).
- This survey shows that significantly ($p < 0.05$) different levels of muscle ChE activity were found in populations of Flounder both between and within estuaries.
- Compared with fish from the reference estuary, which exhibited the assumed 'normal' levels, the majority of those from other sites were showing >40% muscle ChE activity reduction.
- Organophosphate and carbamate pesticides were found in all sampled estuaries except the reference site (see table 2), which suggests these compounds were probable contributors to the observed neurotoxic effect.
- There was no correlation between measurements of enzyme biomarkers and somatic indices, with the exception of female fish from the Tyne estuary.

References

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