THE VIVIPAROUS BLENNY (*Zoarces viviparus*) AS A BIOINDICATOR OF CONTAMINANT EXPOSURE: APPLICATION OF BIOMARKERS OF APOPTOSIS AND DNA DAMAGE

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Introduction

The viviparous blenny (*Zoarces viviparus*), also known as eelpout, is considered a suitable biomonitor for use in European estuarine and coastal waters (Figure 1). We have previously shown that *Z. viviparus* from the industrialised Tyne estuary (UK) display certain histopathologies (e.g. ovotestis and nuclear/cellular pleomorphism), which are associated with contaminant exposure (Stentiford *et al.*, 2003). Furthermore, the prevalence of these pathologies was higher than in fish collected from a less contaminated reference site (the Alde estuary, UK). In this present study, tissue samples were collected from *Z. viviparus* from the Tyne and Alde estuaries and analysed for DNA adducts using the ^{32}P-postlabelling assay and for histopathology.

Figure 1: The viviparous blenny (*Zoarces viviparus*)

Figure 2: Map of the UK showing the location of the Tyne and Alde estuaries

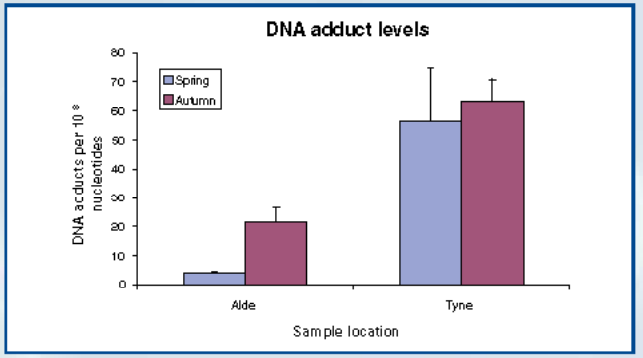

Figure 3: Mean ± SE Levels of hepatic DNA adducts (n = 4 - 5) 4 fish per pooled sample

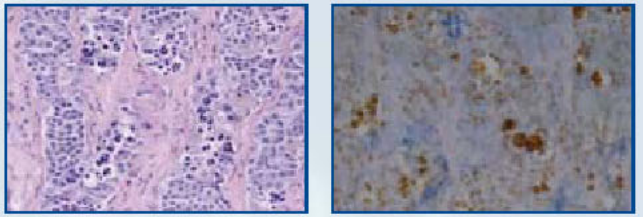

Figure 4: H&E and TUNEL labelled spermatonial cells

Materials and Methods

Sample Collection

The industrially impacted Tyne along with the relatively uncontaminated reference site, the River Alde (Figure 2) were chosen based on previous research indicating contamination differences between the two sites (Matthiessen *et al.*, 1998; Kirby *et al.*, 2000). The River Tyne is known to receive considerable amounts of anthropogenic inputs, including municipal sewage (268 652 m^3 day^{-1}) and industrial effluent (1 162 931 m^3 day^{-1}). In contrast, the River Alde has no recorded industrial inputs and relatively small volumes of municipal wastes enter its waters (145 m^3 day^{-1}). The estuaries were sampled in the spring and autumn of 2000 using a 2 m beam trawl.

DNA adduct analysis

Hepatic DNA adducts were analysed by the ^{32}P-postlabelling assay using butanol enhancement and TLC resolution (Jones *et al.*, 1991).

Apoptosis detection

The terminal deoxynucleotide transferase mediated deoxy-UTP nick-end labelling (TUNEL) assay was carried out following the protocol as set out in the ApopTag® Peroxidase *In Situ* apoptosis detection kit (Cat. No. S7100, Intergen, UK).

Results and Discussion

Previously, we have identified histopathological changes in tissues of *Z. viviparus* indicative of contaminant exposure (Table 1).

Table 1: Occurrence of pathologies detected in gonad and liver samples of *Z viviparus* collected from the Tyne and Alde (adapted from Stentiford *et al.*, 2003). N.A. data not available

		Ovotestis (%)	nuclear/cellular pleomorphism (%)
Tyne	Spring	25%	40%
	Autumn	0	70%
Alde	Spring	0	16.7%
	Autumn	N.A.	N.A.

In this present study, significant differences ($p < 0.05$) were observed between the levels of hepatic DNA adducts detected in Tyne and Alde caught *Z. viviparus* (Figure 3). Interestingly, the levels of hepatic DNA adducts detected in *Z. viviparus* from the Alde were significantly higher in the autumn when compared with samples collected during the spring.

The DNA adduct profiles detected in fish from the Tyne consisted of DRZ's, indicative of exposure to complex mixtures of genotoxins.

Spermatogonial cells in male fish captured from both sites revealed a characteristic pathology whereby affected cells contained condensed and fragmented chromatin profiles.

Further analysis of this tissue revealed that these cells labelled positively with the TUNEL assay, suggesting that they contained DNA with significant quantities of strand breaks. Due to the aberrant morphology of their chromatin, these cells were classified as apoptotic.

While apoptosis has been described in the spermatogonial cells of vertebrates, numerous studies have shown that the relative level of apoptosis can be increased by exposure to sub-optimum environmental conditions (e.g. irradiation, contamination). Further studies on *Z. viviparus* will continue to investigate the prevalence and relative severity of this pathology in populations from different geographical locations. As such, it may provide a potential biomarker for reproductive impairment in male fish.

Acknowledgements

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