

Introduction

Extracellular factors such as hormonal withdrawal, hypertonic stress, radiation and contaminant exposure are known to bring about mutation and apoptosis (programmed cell death) in the testicular germ cells of humans, mice and fish. A previous study by Marty *et al.* (1997) showed that increased gonadal-cell apoptosis of fish was clearly associated with oil exposure as a result of the Exxon Valdez oil spill. We investigated the presence of apoptosis in the gonadal tissues of Atlantic cod (*Gadus morhua*), the viviparous blenny (*Zoarces viviparus*) and the European flounder (*Platichthys flesus*), captured during estuarine and coastal fish health monitoring programmes in the United Kingdom. As part of these assessments, a characteristic pathology of the gonadal tissues, identified as apoptosis, was recorded in male and female fish from several sites.

Sampling & Results

Atlantic cod (*Gadus morhua*) (Figure 1), viviparous blenny (*Zoarces viviparus*), and European flounder (*Platichthys flesus*) were collected from various UK coastal waters and estuarine locations. Gonadal tissues were then processed for standard histology. Three 3µm serial sections were stained with Haematoxylin and eosin (HE), Farley Feulgen (FF) and the terminal deoxynucleotide transferase mediated deoxy-UTP nick-end labelling (TUNEL) assay (Figure 2) using either a fluorescein or peroxidase label to confirm the presence of apoptosis. Slides were examined using a Nikon Eclipse E800 microscope equipped with fluorescent filters. Images were captured using the Lim Lucia G Screen Measurement™ image analysis system (Nikon, UK) and Nikon DXMI200F video camera (Figure 3).



Figure 1: Atlantic cod (*Gadus morhua*)

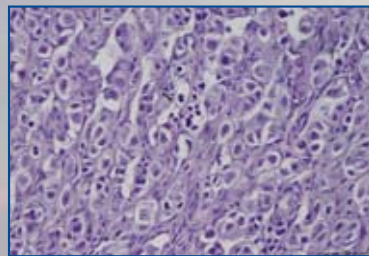


Figure 4: Spermatogonial cells of cod with focus of apoptotic cells. Note the presence of dense nuclear apoptotic bodies (HE)

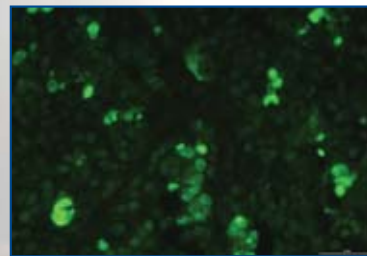


Figure 5: Same fish as Figure 4 labelled with TUNEL Fluorescein. Note the increased sensitivity of the technique for confirmation of apoptotic cells

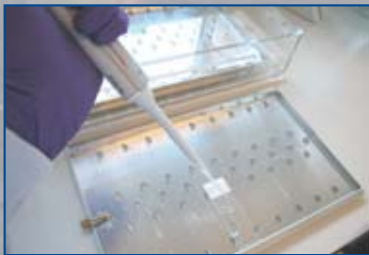


Figure 2: Undertaking the TUNEL assay

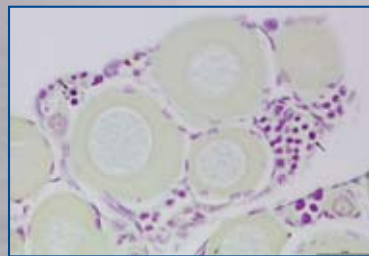


Figure 6: Cod ovary displaying two discrete areas of apoptotic oogenesis (FF)

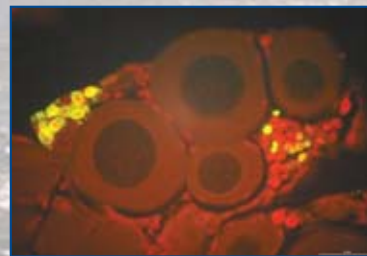


Figure 7: Adjacent serial section labelled using TUNEL Fluorescein

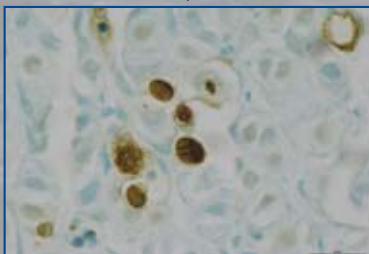


Figure 3: Demonstration of apoptosis of cod spermatogonia using TUNEL Peroxidase

Conclusion

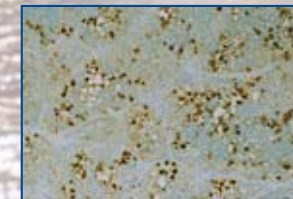
Studies have suggested that increased gonadal germ cell apoptosis can occur as a result of contaminant and other toxicity exposure, perhaps affecting fecundity (Marty *et al.*, 1997). During the current investigation, we have determined that flounder exhibit varying degrees of gonadal germ cell apoptosis. Using image analysis, we are currently developing an index to grade the extent of this condition in the gonad of fish captured from different sites. Although the possible effect of apoptosis needs to be determined, we propose that the rate of apoptosis may be used as a suitable reproductive biomarker of contaminant exposure. In addition, the possible effects of apoptosis of gonadal germ cells on fecundity needs to be assessed.

References

- Bignell, J.P., Feist, S.V. & Stentiford, G.D. Apoptosis in the reproductive organs of estuarine and coastal fish from UK waters (in preparation).
- Marty, G.D. *et al.* (1997). Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. *Canadian Journal of Zoology* 75, 989-1007.



Clyde



Mersey



Southampton

Figure 8: Relative differences in the level of apoptosis in flounder testis sampled from Mersey > Clyde > Southampton sites (TUNEL Peroxidase)

Acknowledgement

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