Immune parameters of plaice, *Pleuronectes platessa*, L. along a sewage sludge gradient in the Firth of Clyde, Scotland

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Plaice were caught at five stations varying in distance from the sewage sludge dumping site at Garroch Head, in the Firth of Clyde, Scotland and a number of physical and immunological parameters monitored. Significant intergroup differences were apparent in condition factor, hepatosomatic index, serum protein concentration, serum lysozyme, serum immunoglobulin (Ig), liver vitamin E, kidney leucocyte respiratory burst activity and kidney leucocyte bactericidal activity. Of these parameters, the hepatosomatic index, serum lysozyme, serum Ig and kidney leucocyte bactericidal activity showed a negative correlation with distance from the dump site (that is, were highest at the dump site); serum protein and liver vitamin E showed a positive correlation. Factors found not to vary between the groups included the spleen index, serum antiprotease activity and kidney leucocyte phagocytic activity. These findings are discussed in relation to recent experimental data on the effects of sewage sludge exposure on the immune system of fish.

Keywords: immunity; plaice; sewage sludge; Firth of Clyde.

Introduction

The hypothesis that chronic exposure to sublethal levels of contaminants in the environment may predispose fish to infectious disease is well known (Sindermann, 1979; Moller, 1985; Watermann and Kranz, 1992) but has been difficult to verify, partly due to a lack of diseases exclusively associated with contaminants (McVicar et al., 1991). Thus, diseases monitored have a natural variability in the absence of contaminants. This has been a complicating factor in studies looking for a relationship between disease prevalence and exposure to sewage sludge contamination in the North Sea, where no clear trends have been found (McVicar et al., 1988, 1991). The immune system, which influences disease susceptibility, is also considered to be a particularly sensitive biological parameter to monitor following exposure to contaminants (Dean et al., 1982) and many studies on mammals have shown the modulating effects of so-called immunotoxins

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(Wong et al., 1992). Studies carried out on fish 'naturally' exposed to contaminants have supported this idea and have shown that a variety of immune parameters are modulated (Faisal et al., 1991a; Seeley and Weeks-Perkins, 1991). However, these studies have been predominantly confined to the effects of exposure to hydrocarbons in the highly polluted lower Chesapeake Bay region.

In controlled laboratory experiments, recent studies have shown that the immune system of the fish is modulated by exposure to sewage sludge, using exposure times of approximately 3 months (Secombes et al., 1991, 1992) and that such fish have an increased disease susceptibility (Bucher and Hofer, 1993). Whilst such data indicate that exposure to the particular conditions employed can influence fish health, it is clearly difficult to extrapolate them to the environmental situation, where fish may not be exposed to a constant contaminant dose and the length of exposure may vary enormously. Thus, despite the potential problems of using feral fish, the wealth of data obtained in the above experiments combined with the lack of studies evaluating the effects of 'natural' exposure to sewage sludge on a range of immunological parameters, clearly warrants an environmental study. The present work, evaluating the immune responses of plaice (*Pleuronectes platessa*) caught at different sites along a sewage sludge gradient in the Firth of Clyde, Scotland, addresses this issue.

**Materials and methods**

**Collection of fish**

Plaice (*P. platessa*), between 21.5 and 25 cm in length, were caught by trawl in the Firth of Clyde in May 1990, at varying distances from the sewage sludge dump site at Garroch Head (Fig. 1). This included collection of fish from the dump site itself (site 1), 2 km (site 2), 9 km (site 3) and 15 km (site 4) from the dump site and from a clean site to the south-west of the Isle of Arran (site 5) ~40 km away. Sexes were distributed equally at each site, other than site 4, where the catch was entirely female. Fish were collected from only one site each day, landed between 18.00 and 20.00 h and kept overnight in seawater tanks at the Universities Marine Biological Station, Millport. The following morning ten fish were killed, weighed/measured, blood samples taken, livers and spleens weighed and livers frozen and kidneys removed for immunological analysis. The condition factor (weight per length$^3 \times 100$), hepatosomatic index (g liver weight $\div$ g body weight $\times 100$) and spleen index (mg spleen weight $\div$ g body weight) were all calculated.

**Serum assays**

After overnight storage at 4°C the blood samples were centrifuged, the serum collected and frozen in aliquots at $-70°C$. Serum samples were analysed for protein and immunoglobulin (Ig) content and for lysozyme and antiprotease activity. Serum protein levels were determined by the Bradford (1976) assay. Ig levels were determined by a sandwich ELISA assay using rabbit anti-dab Ig, as described by Secombes et al. (1991) for dab. The only difference was that the peroxidase-labelled anti-dab Ig was diluted 1 : 1000 instead of 1 : 4000, due to the lower reactivity of this antibody with plaice Ig. Since purified plaice Ig was not available to make a standard curve, the results were expressed as the optical density (OD) at 450 nm.