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Hepatic metallothionein and total oxyradical scavenging capacity in Atlantic cod *Gadus morhua* caged in open sea contamination gradients

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Abstract

Biological effects monitoring has seldom been undertaken in offshore pelagic environments. Cages containing hatchery-reared Atlantic cod *Gadus morhua* were deployed on expected contamination gradients, along a transect from the River Elbe in the German Bight, and in the vicinity of an oil field in the North Sea (Statfjord). Six weeks later, the cod were retrieved and samples taken for a range of biological effects techniques. In this study, metallothionein (MT) and total oxyradical scavenging capacity (TOSC) were measured in liver samples from the caged cod, together with metals (as a measure of bioaccumulation). Both MT and TOSC were highest in cod from the German Bight. In the Statfjord samples MT and TOSC decreased with distance from the oil platform indicating induction in response to anthropogenic sources. The bioavailability of metals appears to be a major factor in MT synthesis, and the measurement of MT and associated metals is shown to be a useful tool for biological exposure and effects monitoring in pelagic systems. There also appears to be a strong linkage between MT and TOSC levels, indicating overlapping capabilities as stress biomarkers. Results suggest that in addition to its role as a specific indicator of metal exposure, MT in cod could act as a more general biomarker of oxidative stress under certain conditions.

Keywords: Metallothionein; TOSC; Metals; Cod; North Sea

1. Introduction

It is difficult to predict the extent to which pelagic fish are exposed to contaminants due to inherent problems of fish mobility, water movement and dilution factors. The method of caging fish *in situ*, and of measuring bioaccumulation and biomarkers in native populations has proved to be effective in determining the extent, and effect of exposure to contaminants in freshwater, estuarine and coastal environments: endpoints measured have included body burdens, MT induction, elevated ethoxyresorufin-*O*-deethylase (EROD) activity, and DNA strand breaks and adducts (Langston et al., 2002; Klaverkamp et al., 2006; Roch and McCarter, 1984; Orrego et al., 2006; Winter et al., 2004). In the open sea, determining the bioavailability and biological significance of contaminants in fish is more problematic, and although sophisticated models can attempt to calculate dispersion, dilution and partitioning processes, the ultimate fate of contaminants, and levels of exposure for mobile fish populations remains largely unknown.

This study was part of the ICES BECPELAG (biological effects of contaminants in pelagic ecosystems) project, a workshop focussing on detection of contaminant effects in two North Sea areas, the German Bight – a coastal area, and Statfjord – an area in the vicinity of an oil rig. For many years, the North Sea has received a significant load of contaminants from anthropogenic activities. These enter the system via point-source coastal inputs (domestic and industrial effluents) and indirect sources (principally, in the German Bight, the Rivers Elbe, Ems, Weser and Eider). Additionally, offshore oil and gas production are the source of large inputs of produced water. Contaminants in both areas include inorganic and organic compounds (e.g. metals, hydrocarbons and surfactants) details of which are described in Schmidt, 1985; Schonfeld et al., 1990; Hardy and Cleary, 1992; MURSYS, 2000; Utvik and Gärtner, 2006; McIntosh et al., 2006.

Assessment of the ability of the metallothionein (MT) bioassay to detect biological effects of such inputs in pelagic systems was one of the objectives of the current study. Research has to date provided evidence of MT involvement in metal detox-

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ification, homeostasis of Zn and Cu, and protection against radiation-induced DNA damage and oxidative stress (Roesijadi, 1992; Olsson et al., 1987; Cai and Cherian, 2003; Kling et al., 1996; Kiningham and Kasarskis, 1998). MT has been purified and characterised from numerous fish species, and is thought to be ubiquitous among teleosts, chiefly in detoxification and storage organs such as liver and kidney (Klaverkamp et al., 1984; De Smet et al., 2001), but also to a lesser extent in gills, muscle (De Smet et al., 2001; Dang et al., 2001; Olsson et al., 1989; Hogstrand et al., 1995; own unpublished data), brain (Scudiero et al., 2000), intestine (Shears and Fletcher, 1984) and gonad (Kling et al., 1996).

The induction of MT in teleosts, and its capacity to bind metals, has been applied in monitoring of various polluted aquatic ecosystems. For example, brown trout *Salmo trutta* caged in a Cd/Zn contaminated river have been shown to respond by production of MT (both protein and mRNA) (Hansen et al., 2006); similarly, MT levels in perch *Perca fluviatilis* from a (Cd) contaminated watercourse were found to be correlated with Cd body burdens (Olsson and Haux, 1986). Rainbow trout *Oncorhynchus mykiss* caged in lakes with differing metal concentrations also showed a strong correlation between MT and Zn concentrations (Roch and McCarter, 1984). In nearshore marine environments (Forth Estuary) highest levels of hepatic MT induction in flounder *Platyichthys flesus* coincided with sites exhibiting the most metal-contaminated sediments (Sulaiman et al., 1991), as was the case in eel *Anguilla anguilla* from the Thames Estuary (Langston et al., 2002). MT induction in marine fish has also occasionally been used to monitor the effects of episodic events such as dredging: thus, elevated MT gene expression in eelpout *Zoarces viviparus* was detected in response to an increase in metals remobilized during extensive removal of sediments in Goteborg harbour (Sturve et al., 2005). Changes in sub-cellular partitioning of metals can provide valuable additional information on metal exposure and indicates whether the ability of an organism to maintain metal homeostasis is being compromised. Thus, Cu in the MT fraction has been shown to increase with metal contamination in rainbow trout populations (Roch et al., 1982). Likewise in yellow perch *Perca flavescens*, caged in a Cd-polluted lake, the MT fraction became an increasingly significant sink for hepatic Cd, playing a progressively more important role in sub-cellular regulation of Cd over exposure time (Kraemer et al., 2005).

Examining metallothionein levels and metal-binding characteristics in fish has, therefore, been applied successfully to field studies in rivers, estuaries and lakes, and can serve as an early indication of contaminant stress, providing valuable information regarding the extent and possible consequences of chronic exposure. However, to date few studies have investigated the role of metallothionein as a 'biomarker' in pelagic marine systems, where chronic exposure to contaminants, even in a large water mass, may have the potential to induce sub-lethal effects.

Over recent years, there has been a growing interest in the effect of contaminant-induced oxidative stress on aquatic biota. Increases in markers of oxidative stress in aquatic organisms, or in cell cultures derived from them, have been reported after laboratory exposure to hydrocarbons and PAHs (Steadman et al., 1991; Peters et al., 1996; Winzer et al., 2000). Metals, such as, e.g. Cd and Ni, are also inducers of oxidative stress and may interfere with the anti-oxidant enzymatic defence system in fish (Rodríguez-Aziza et al., 1993; van der Oost et al., 2003; Romeo et al., 2000). Specific components of the anti-oxidant system (including superoxide dismutase, glutathione peroxidase, glutathione reductase and levels of total glutathione) can be measured as biomarkers of pollutant-mediated oxidative stress (Regoli et al., 2002), although their ecological relevance may be restricted (Company et al., 2006). The total oxyradical scavenging capacity (TOSC) assay overcomes this criticism since it measures the overall capacity of a tissue to absorb different reactive oxygen species (ROS), and can provide a quantifiable value of susceptibility to oxidative stress (Regoli, 2000; Regoli and Winston, 1999). In one of the few field trials with fish, Regoli et al. (2002) found significantly higher TOSC values in red mullet *Mullus barbatus* from an offshore dredge-disposal site, compared to those from remote areas. An increase in TOSC may therefore characterise chronic contamination, though in acute conditions suppression is more likely. Reduced TOSC has been shown to correlate with DNA damage (Regoli et al., 2003; Frenzilli et al., 2001) and with lysosomal stability (Regoli, 2000) and it may therefore be a precursor of higher order effects. Since metal toxicity can involve the production of ROS (Watanabe et al., 2003; Manaca et al., 1994; Zhong et al., 1990), links between oxidative stress and metals, and MT and TOSC responses might be expected, though few studies have examined this possibility.

In an attempt to address this uncertainty, farmed cod, with low previous exposure to contaminants, were used in caging experiments in the North Sea, along expected contamination gradients, and biomarker studies performed following deployment. The current study was designed to investigate levels of MT and associated metals, and TOSC, in liver samples. The liver was the chosen organ for analysis because of its central role in the metabolism, sequestration, and excretion of xenobiotics; it is also important in digestion and storage, and in reproduction through the production of the yolk precursor protein vitellogenin (Hinton and Lauren, 1990; Köhler, 1991).

2. Materials and methods

2.1. Sample collection

In April 2001, cages were deployed at stations in relation to suspected trends in contamination (Fig. 1), i.e. along an axial transect from German Bight (sites G1–4), and at 0.5, 2, and 10 km from an oil rig in the Statfjord oilfield (Statoil Rig 'B) (sites S1–3) plus a reference station SSE of the oilfield (site S4). The cages were deployed at 12–15-m depth, and each contained 50 hatchery-reared Atlantic cod (*Gadus morhua*), from the Parisvatn Field Station in Norway. A battery and light were attached to each cage to attract plankton (krill, etc.) providing food for the caged fish. In June, 6 weeks later, the cages were retrieved from all sites, weight and length measurements of each fish were taken and condition noted. The fish were sacrificed and the livers excised, cut into portions and frozen in liquid nitrogen. Samples were shipped to the MBA laboratory on dry ice.