**Benthic Fish as Sentinel Organisms of Estuarine Sediment Toxicity**

Mark G. J. Hartl

Department of Zoology & Animal Ecology, Environmental Research Institute, University College Cork, Lee Maltings, Prospect Row, Cork, Ireland. E-mail: m.hartl@ucc.ie

Keywords: Ecotoxicology, fish, sediment, estuaries

**Abstract.** The high productivity of estuarine environments offers and sustains important habitats for many fish species but also attracts an ever increasing level of human activity, of both commercial and recreational nature. Consequently, estuarine sediments are often the most heavily polluted marine environments, owing to communal and industrial effluent discharge and pesticide input from agricultural runoff and antifouling agents. With the relative improvement of estuarine water quality in recent years, the focus in aquatic ecotoxicology has increasingly turned to monitoring estuarine sediments, as they may not only act as sinks but also as secondary sources of persistent pollutants. The present paper offers a concise selection of examples of biomarkers for a wide range of toxic pollutants commonly found in estuarine sediments and the fish species employed as sentinel organisms in sediment toxicity assessments. The applications, advantages and limitations of specific biomarkers as diagnostic tools are presented and discussed.

**Introduction**

The term 'ecotoxicology' was first introduced by the toxicologist Prof. Truhaut in the late 1960s, when it was considered a sub-discipline of medical toxicology. Since then, ecotoxicology has developed into a scientific discipline in its own right, describing not only effects of exposure to chemicals and radiation, but also the environmental fate of contaminants. Toxic pollutants often cause characteristic

Bright, M., P.C. Dworschak & M. Stachowitsch (Eds.) 2002: The Vienna School of Marine Biology: A Tribute to Jörg Ott. Facultas Universitätsverlag, Wien: 89-100
responses in the affected organism, commonly known as 'toxicological endpoints' or 'biomarkers'. A biomarker, as defined by Depledge et al. (1993), is "...a biochemical, [genetic] cellular, physiological or behavioural variation that can be measured in tissue or body fluid samples or at the level of the whole organism (either individuals or populations), that provides evidence of exposure and/or effects of one or more chemical pollutants (and/or radiation)". Biomarkers are powerful tools for detecting the impact of exposure to sublethal concentrations of a given substance or complex chemical mixtures, enabling the evaluation of less obvious effects on organisms. In aquatic ecotoxicology the use of biomarkers has traditionally been applied to the exposure of sentinel organisms or in vitro test systems to pollutants in aqueous solutions or suspensions. These approaches have been instrumental in providing guidelines for legislative measures aimed at reducing the impact of anthropogenic activity on marine and freshwater environments. In recent years, however, the relative improvement of water quality in many areas and the recognition that sediments may serve as sinks and secondary sources for many persistent chemicals (Harris et al., 1996) has shifted the focus of ecotoxicological studies towards sediments and the potential deleterious effects persistent pollutants have on benthic ecosystems (Anderson et al., 1996).

One ecosystem that has attracted a significant amount of attention is estuaries. Generally, estuaries are areas of high productivity, which gives them not only ecological significance but also attracts economic interest. With the increasing pressure of anthropogenic activity from agricultural runoff, industrial effluent, airborne pollutant input, biocidal agents from antifouling paints etc., and the recent effects on maricultural industries, it is not surprising that many aquatic ecotoxicology studies have been aimed at monitoring the health of estuarine ecosystems (Langston et al., 1990; Chapman & Wang, 2001). Estuaries are areas of constant change caused by varying meteorological and hydrographic factors that regulate the physicochemical properties of estuarine water and sediments. In estuaries, especially those exposed to strong tidal action, parameters such as, pH, salinity, oxygen concentration and temperature fluctuate. These are important abiotic factors dictating not only the chemical ‘species’, but – together with the aqueous solubility of the compound, redox, affinity for sediments, organic carbon content and sediment mineral constituents – the sorption behaviour and in turn the bioavailability of many pollutants (Chapman & Wang, 2001). In addition, re-suspension from bioturbation and turbulence, or by storms, tides and anthropogenic activity, represent some of the physical means of releasing pollutants from sediments and their re-introduction into the sediment-water interface (Watanabe et al., 1997).

The sediment-water interface is an important habitat for benthic or demersal fish, in search for food or shelter. The constant input of particle-associated pollu-
tants, due to sedimentation, brings about potentially high concentrations of contaminants in sediments and therefore warrants particular attention from ecotoxicologists. Toxic metals and organic compounds can affect living organisms at various levels of organisation, inducing a biomarker response. It is therefore necessary to study the toxic effects of pollutants at each level and to incorporate additional parameters, such as season, the developmental stage and sex of the test organism, in order to determine the quantitative relationship between pollutant toxicity, the organism, population sustainability and ecosystem effects.

This paper is not a comprehensive review of aquatic ecotoxicology – there are excellent reviews available in the literature (Calmano & Förstner, 1996; Ingersoll, 1997; Luoma & Ho, 1998) – but rather a concise selection of some examples of various benthic fish species used as sentinels for a range of toxic pollutants commonly found in estuarine sediments and their respective biomarkers. The applications, advantages and limitations of fish biomarkers as diagnostic tools are considered. Furthermore, this paper is intended to provide the reader with a quick reference facility, not only for selecting a practical and ecologically relevant suite(s) of biomarkers for the pollutant of interest, but also for identifying potential needs for further research in the area of fish-related sediment-associated aquatic ecotoxicology.

**Whole sediment toxicity assays**

Whole sediment toxicity assays are widely used for ecological impact assessments of anthropogenic activity on fish. The test organism can either be observed in its natural habitat, exposed to field-collected sediments in the laboratory or transplanted from a "clean" environment to a polluted one in caging experiments (e.g. along a transect from a point source). Apart from a few examples (e.g. TBT and imposex in gastropods), the biomarker response mainly indicates the presence of a substance class, rather than a specific chemical. Polluted sediments regularly contain complex chemical mixtures, giving rise to the potential problem of synergistic and/or antagonistic effects (Eggens *et al.*, 1995). This makes establishing causality in the field particularly difficult and therefore most evidence of biomarker responses recorded in whole sediment toxicity assays are of a correlative nature. Whole sediment toxicity tests without specific biomarkers (Crane *et al.*, 2000) can highlight the route of exposure and, more importantly, the bioavailability of sediment-associated pollutants to a given organism. Examples of whole sediment assays, the main pollutants involved and the biomarker responses induced in selected benthic fish species are listed in Table 1.
In vivo tests with spiked sediments

A common approach to ecotoxicological studies is in vivo experiments in the laboratory conducted with either clean field-collected or artificial sediments spiked with the compound(s) of interest. The obvious benefit of such studies is that a cause-and-effect relationship can be demonstrated under controlled conditions, without the potential interactions of other compounds, thereby minimising synergistic effects.

The ecological relevance of laboratory experiments depends on the experimental design and has clear limitations when applying the results to a field scenario (Burton, 1991). These models, however, contribute important information to the understanding of the potential toxicological impact of contaminated sediments on benthic organisms, including fish, and the presence of pollutant metabolites can help elucidate the biochemical pathways of the toxic effects involved. Recent studies using in vivo tests involving spiked sediments are listed in Table 2.
Table 1c. Examples of whole sediment assays using fish (Pleuronectiformes). For abbreviations see appendix.

<table>
<thead>
<tr>
<th>sentinel organism</th>
<th>measured chemicals</th>
<th>biomarker</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citharichthys stigmaeus</td>
<td>PAHs, PCBs, metals, PAHs, pp’DDE</td>
<td>P4501A (CYP1A), EROD</td>
<td>Gunther et al. (1997)</td>
</tr>
<tr>
<td>Limnada limnada</td>
<td>PCBs, Zn, Cu, Cd</td>
<td>DNA strand breaks, MTs</td>
<td>Everaarts (1995), Hylland et al. (1992)</td>
</tr>
<tr>
<td>Pleuronectes vetulus</td>
<td>PAHs, PCBs, PAHs</td>
<td>hepatic lesions, plasma levels of oestradiol, vitallogenin levels, reproductive success</td>
<td>Myers (1998), Rice et al. (2000)</td>
</tr>
<tr>
<td>Pleuronectes americanus</td>
<td>PCBs, PAHs, DTT</td>
<td>histopathological lesions, hepatic lesions</td>
<td>Johnson et al. (1993a), Myers et al. (1998)</td>
</tr>
<tr>
<td>Platichthys stellatus</td>
<td>PCBs, DDTs, PAHs, Chlordanes, Dieldrin, PAHs</td>
<td>hepatic lesions</td>
<td>Stehr et al. (1997), Myers et al. (1998)</td>
</tr>
<tr>
<td>Platichthys flesus</td>
<td>PAHs, PCBs, Cd, Hg, PAHs, PCBs</td>
<td>hepatic lesions and neoplasia, FACs, AST</td>
<td>Beyer et al. (1996), Besselink et al. (1998)</td>
</tr>
<tr>
<td>Rhombosolea tapirina</td>
<td>TCDD, PCBs, PAHs</td>
<td>P450 1A (CYP 1A); EROD</td>
<td>Grinwis et al. (2000)</td>
</tr>
</tbody>
</table>

Table 1d. Examples of whole sediment assays using fish (Cypriniformes & Cyprinodontiformes). For abbreviations see appendix.

<table>
<thead>
<tr>
<th>sentinel organism</th>
<th>measured chemicals</th>
<th>biomarker</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptychocheilus oregonensis</td>
<td>PAHs, PAHs, dioxines</td>
<td>DNA damage, FACs</td>
<td>Curtis et al. (1993)</td>
</tr>
<tr>
<td>Cyprinus carpio</td>
<td>PAHs</td>
<td>P450 1A (CYP 1A); EROD, GSTs, GSH</td>
<td>Machala et al. (1997)</td>
</tr>
<tr>
<td>Fundulus heteroclitus</td>
<td>Cd, Pb, Cu, Hg, Zn</td>
<td>DNA damage - single strand breaks, behaviour</td>
<td>Pandrangi et al. (1995), Weis (2001)</td>
</tr>
</tbody>
</table>

**Important substance classes and relevant biomarkers**

**Biomarkers of metal contamination**

Metals exist in the environment as various inorganic species and/or as organo-metallic compounds. As outlined above, the speciation of most metals affects sorption behaviour and in turn dictates bioavailability to benthic organisms (Bryan, 1985). Bioavailability of sediment-associated metals can also vary greatly between
different taxa. For instance, Hylland et al. (1996) found sediment-associated metals to be largely unavailable to caged flounders, whereas bivalves are known to concentrate large amounts of metals in their tissues (Goldberg, 1975). Nevertheless, there are well-defined biomarkers of inorganic metal exposure in fish, including the induction of metallothioneins (Hylland et al., 1992) and lysosomal destabilization (Fent & Hunn, 1996).

Organometallic compounds are often highly lipophilic and hence more readily bioavailable. Biomarkers of exposure to organometallic pollutants, such as TBT, have been associated with failing membrane integrity, which can either be directly measured as membrane lesions (Grinwis et al., 1998) or are more subtle and manifest themselves as physiological abnormalities (Hartl, 2000; Hartl et al., 2000; Hartl et al., 2001a; b, c, d).

### Biomarkers of polycyclic aromatic compounds

Polycyclic aromatic compounds (PACs) constitute a large group of naturally occurring and synthetic persistent organic chemicals found in the environment. They include polycyclic aromatic hydrocarbons (PAHs) and polychlorinated aromatic compounds (PCAs), such as polychlorinated naphthalenes (PCNs), polychlorinated biphenols (PCBs), and dioxins. Major entry routes of PACs into the aquatic environment involve biosynthesis, spillage and seepage of fossil fuels, discharge of domestic and industrial wastes, atmospheric input and continental runoff. The hepatic biotransformation enzyme cytochrome P4501A, a co-binding protein of the mixed-function oxidinase (MFO) system, is specifically induced by PACs with a planar configuration. 7-ethoxyresorufin O-deethylase (EROD) is catalysed by P4501A (CYP1A). This makes the activity of EROD a good indicator of MFO induction and a widely employed biomarker for organic pollution (Lee, 1981; Livingstone, 1991; Kirby et al., 1999). Its induction is involved in chemical carcinogenesis via catalysis of the covalent binding of organic contaminants to DNA, forming DNA-adducts (Livingstone, 1993). A correlation between

<table>
<thead>
<tr>
<th>sentinel organism</th>
<th>chemicals spiked</th>
<th>biomarker</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Platichthys flesus</em></td>
<td>organotins</td>
<td>ATPase activity, gill morphology, membrane permeability, Na⁺ flux, osmotic stress, blood osmolality</td>
<td>Hartl et al. (2001c, d)</td>
</tr>
<tr>
<td><em>Pleuronectes vetulus</em></td>
<td>PCBs, PAHs</td>
<td>P450 1A (CYP 1A); EROD</td>
<td>Rice et al. (2000)</td>
</tr>
</tbody>
</table>

Table 2. Examples of spiked sediments in ecotoxicological assays using fish. For abbreviations see appendix.
levels of enzyme induction and concentration has been demonstrated, enabling a crude impact assessment of organic pollution (Wong et al., 2000). P4501A induction is most pronounced in vertebrates and to a lesser extent in some invertebrates (Lee, 1981), where other biomarkers have proven to be more reliable.

Conclusions

Why use biomarkers or toxicity tests? Chemical analysis provides baseline information on the occurrence of potentially toxic pollutants in the environment, but fails to predict synergistic, additive or antagonistic effects, that may give an important measure of potential biological effects. Biomarkers on the other hand can detect direct and indirect effects of sublethal concentrations of toxic pollutants and offer additional biologically and ecologically relevant information – a valuable tool for the establishment of guidelines for effective environmental management (Long et al., 2000).

However, not all benthic organisms are suitable as sentinels for sediment toxicity. Some may be more susceptible to certain pollutants than others (Driscoll & McElroy, 1996). Furthermore, as indicated in the introduction, certain pollutants may not necessarily cause a measurable response at every level of organisation in a particular organism: a seemingly negligible impact on one level of organisation might lead to serious knock-on effects on others (Kurelec, 1993). Biomarkers should therefore ideally be employed as part of an integrated programme of pollution monitoring, involving general measurements of biological damage and animal health in a variety of taxa as well as analysis of chemical contaminants in the biota and environment.

Acknowledgements

Thanks to J. O’Halloran for helpful comments on an earlier draft of this manuscript.

References


Gadagbui, B.K.M. & A. Goksoyr, 1996: CYP1A and other biomarker responses to effluents from a textile mill in the Volta River (Ghana) using caged tilapia (Oreochromis niloticus) and sediment-exposed mudfish (Clarias anguillaris). Biomarkers, 1: 252-261.


michogs (Fundulus heteroclitus) as a behavioral biomarker for contaminants in estu-

Willett, K. L., S. J. McDonald, M. A. Steinberg, K. B. Beatty, M. C. Kennicutt & S. H. 
Safe, 1997: Biomarker sensitivity for polynuclear aromatic hydrocarbon contamination 
in two marine fish species collected in Galveston Bay, Texas. Environ. Toxicol. 

Ecotoxicological assessment of persistent organic and heavy metal contamination in 

cytochrome P4501A1 gene in gill, intestine and liver of tilapia exposed to coastal sed-

Zapata-Perez, O., R. Sima-Alvarez, E. Norena-Barroso, J. Guemes, G. Gold-Bouchot, A. 
Ortega & A. Albores-Medina, 2000: Toxicity of sediments from Bahia de Chetumal, 
Mexico, as assessed by hepatic EROD induction and histology in nile tilapia 

Appendix: Abbreviations used in tables

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>DDT</td>
<td>dichlordiphenyltrichloro-ethane</td>
</tr>
<tr>
<td>EROD</td>
<td>7-ethoxyresorufin O-deethylase</td>
</tr>
<tr>
<td>FAC</td>
<td>fluorescent aromatic compounds</td>
</tr>
<tr>
<td>MTs</td>
<td>metallothioneins</td>
</tr>
<tr>
<td>PAH</td>
<td>polyaromatic hydrocarbons</td>
</tr>
<tr>
<td>PCB</td>
<td>polychlorinated biphenyls</td>
</tr>
<tr>
<td>PDH</td>
<td>propionaldehyde dehydrogenase</td>
</tr>
</tbody>
</table>
| pp'DDE       | para-para dichlorodiphenyldichloro-
                        ethylene |
| RGS          | Reporter Gene System                  |
| SOD          | superoxide dismutase                  |
| TCDD         | 2,3,7,8-tetrachlorodibenzo-p-dioxin   |
| UDP-GT       | uracilidiphosphate-glucuronyl transferase |