International Workshop on Drilling Fluids,

Ecotoxicology and Biodegradation

8-9 October 1998

Edited by

Dr Jill Woodworth
Aquatic Science Research Unit
Muresk Institute of Agriculture
Curtin University of Technology

1857-2-99

Use of Fish Biomarkers in Field Monitoring - Application to the Australian Oil and Gas Industry

M.M. Gagnon, PhD, School of Environmental Biology, Curtin University of Technology, Perth, Western Australia 6845. email. m.gagnon@curtin.edu.au

Abstract

Impacts of industrial activities on the environment have traditionally been estimated through contaminant loads in water, sediment and biota. Due to the high rate of metabolism and depuration of hydrocarbons by fish, contaminant body burden generally does not provide the oil and gas industry with a useful indicator of exposure of fish to petroleum compounds.

Biochemical markers in fish can provide the oil and gas industry with an evaluation of sublethal effects of contaminant on fish populations. The presence of contaminants in the ecosystems may cause perturbations at various levels of biological organization, and biochemical markers may be selected to identify disturbances from the cellular to the population dynamic levels. Biochemical markers of exposure and of effects applicable to this class of contaminants include: the mixed function oxygenases (MFOs) detoxification enzymes in the liver, indicative of exposure of fish to several xenobiotics; sorbitol dehydrogenase in the blood, characteristic of liver injury caused by chemicals; bile metabolites, indicative of excretion capabilities of the organism; metabolic enzymes, representative of disturbances in basic metabolism; stress proteins, general integrator of environmental stresses applied on an organism; and DNA adducts, correlated to the prevalence of neoplasia in fish. These biochemical markers may advantageously be complemented by histological and physiological markers reflecting impacts of contaminants at higher levels of biological organization.

Although field studies pose their own challenges, they provide a measure of biological response reflecting the integration of chronic exposure to anthropogenic and to environmental stressors; biological responses measured in the field also integrate natural compensation mechanisms in place to return perturbed environments to unperturbed levels.

Introduction

Over the past centuries, Australia's ecosystems have been deeply perturbed by the increasing human population inhabiting this continent. The impacts of human settlement and exploitation of the land and sea can be observed through the ever-increasing list of threatened or extinct species associated with profound disturbances in the health of ecosystems. In other words, disappearance of species follows disruption of ecosystem integrity - the capability of the system as an entity to maintain equilibrium while absorbing perturbations. Our understanding of mechanisms involved in maintaining this equilibrium is so limited that by the time impairment in equilibrium is established, the collapse of the system is imminent. To prevent such extreme to occur, diagnosis tools analogous to the ones used in human clinical pathology have been adapted to ecological studies. By applying a suite of physiological and biochemical markers of exposure in one or more indicator species, environmental managers are provided with a

holistic evaluation on the environmental situation and can implement measures to prevent further deterioration and favor re-establishment of ecological equilibrium.

The monitoring of xenobiotics in the environment has become an increasingly important issue in recent years, as the persistence and biological activity of some of these compounds have been recognized (Holdway et al. 1995). The complete understanding of an ecosystem is of course an unattainable goal; in addition, the relationship between the stress and the impact may be very complex. As we are moving towards a preventive approach to ecosystem health, it becomes necessary for the environmental managers to use early warning indicators of environmental deterioration. Biochemical indicators of ecosystem health must have the potential to indicate lost of equilibrium beyond the capability of the system to recover from occasional anthropogenic or natural stress. It had recently become possible to detect some pathological changes in indicator organisms; fish have often been used in this respect. Fish are present in most waters and their welfare is relevant to the public, to environmental control and to sport and commercial fisheries. However, because of the high rate of metabolism and depuration of several xenobiotics by many species of fish, direct measurement of tissue concentrations generally does not provide a useful indicator of exposure of organisms to contaminants (Collier et al. 1996). Morever, contaminant body burden does not inform on the type and/or intensity of adverses effects on the organism. Biological tools attesting the uptake and depuration of xenobiotic compounds are used to assess exposure and effects on biota. A systematic approach tracking the pathological effects of xenobiotics using these higher organisms may efficiently signal environmental deterioration at an early stage, as well as establishing baseline condition prior exploitation of a site, or indicating the degree of recovery post-exploitation. The severity of a situation, as well as temporal and geographical aspects can be assessed using a selected suite of sensitive biochemical markers of exposure and of effects.

Laboratory exposures, acute or chronic, feature highly controlled environmental conditions; they have the manipulative capability of observing cause-and-effect relationships although their ecological significance is limited to the number of experimental variables examined. To date, laboratory studies performed in Australia were mostly acute toxicity tests, and chronic sublethal studies using Australian marine species are sparse (Evans, 1994). In the other hand, field studies are crucial in identifying induced environmental changes but lack the specificity to pinpoint cause-and-effect relationships (Niimi 1990; Evans 1994). The recent focus on field studies is based on the knowledge that chemical concentrations in aquatic ecosystems are rarely acutely toxic to fish but rather, organisms are usually exposed in a chronic manner. Morever, ecosystems are usually contaminated by complex chemical mixtures in which compounds may interact to produce antagonist, additive and/or synergistic effects on biota. Clearly, complementary investigations relating laboratory and field responses are needed to seize and understand biological effects of contaminants under field situations.

Diagnosis of ecosystem pathology

Any abnormality observed in an ecosystem may signal the onset of induced disturbances, but the challenge is to identify these abnormalities bearing significant long-term implications for the ecosystem. A systemic approach to tracking pathological conditions in field situations involves the selection of indicator organisms and the monitoring

of their health through a suite of biochemical markers of exposure and of effects. Biochemical markers must be sensitive enough at environmentally relevant concentrations, and be able to account for different biological and environmental factors. The relationship between a measured response and one that produce impairment is often difficult to establish; in addition, correlative relationships between a response and a suspected cause do not imply causality, unless detailed controlled studies previously demonstrated a clear association between factors. Field studies are mainly observational and controlled experiments at the ecosystem level demonstrating cause-effect relationships are practically impossible. Thus, evidences relating cause and effect in field studies must be founded primarily on four epidemiological criterias: strength of association, consistency of replication, specificity and coherence. The strength of association refers to the degree to which the supposed cause and outcome coincide in their distribution - that is, the higher the prevalence of a disorder in an exposed population, the more likely it is to be causal. The criteria of consistency must be satisfied by a demonstration of persistence of effects in a variety of habitats - there will always be abiotic and biotic differences between sites, but a measured biological response must consistently be observed in association with the suspected cause. The specificity of an association describes the precision with which the occurrence of one variable predicts another; by selecting specific physiological/biochemical responses, the specificity of association can be demonstrated. Finally, coherence requires that the association between suspected cause and effects is consistent with theory (Dodson, 1991). Each of these criteria must be accounted for in the sampling design, which must include measurement of carefully selected physiological/biochemical parameters (specificity, coherence) at several reference and impacted sites (strength, consistency).

Selection of biological indicator

In the early stages of field investigations, biological indicator (bioindicator) organisms inhabiting the concerned ecosystems have to be selected. It is worthy to mention that the organism(s) selected must be sensitive enough to react to low-level contamination, but robust enough to be present in severely contaminated environments. To be sampled effectively, the bioindicator(s) should be abundant in the studied environment, easily handled as well as relatively sedentary to be representative of the environment in which it is collected. The animal should also be of a large enough size to allow collection of material for all selected biochemical markers - in a previous Canadian study, for example, each adult fish collected was generating 65 data points (see Gagnon et al. 1994, 1995 for this study). In order to take into account various biotic and abiotic factors. it is also necessary to have a basic understanding of the bioindicator's biology. Finally, a desirable characteristic of a bioindicator species is to be ubiquitous in space and time, allowing conclusions of the research to be applied on a large scale.

In Southeast Australia, the southern sand flathead (*Platycephalus bassensis*) have now been identified as a suitable bioindicator species for its desirable characteristics of sensitivity, abundance and ubiquity (Smith and Gagnon, in press). Recent experiments inducing EROD activity through PCB injections showed that hepatic EROD activity in southern sand flathead could be induced by as much as 20 times relative to uncontaminated fish (Fig. 1A), which indicate that this species can be an excellent biomonitoring tool (Smith and Gagnon, in press). Two other species trialed, sixspine leatherjacket *Meuschenia freycinetti* and bluethroat wrasse *Notolabrus tetricus* did not, however, exhibit sensitivity to contaminants and therefore, were declared unsuitable as biological monitoring tools. An

appropriate bioindicator fish species still remain to be identified in Western Australia; because this state is so vast and its coastal environment is composed of a variety of habitats, distinct fish species may have to be selected in order to adapt the study design to the studied environment.

Selection of biochemical markers

Significant alterations in biochemical markers potentially represent significant deleterious impacts at the ecosystem level, because responses at the individual organism level will at some stage result in impacts at the population, community and ecosystem levels of biological organization (McCarthy and Shugart 1990). The delay between the manifestation of biological markers of exposure at the individual level and irreversible impacts at higher levels of biological organization warrants the use of biological markers of exposure as early warning system of environmental deterioration. Biological markers may be measured from the molecular to the ecosystem levels; however, most biological markers of exposure had focussed from the molecular to the organism levels because our understanding of mode of action and toxicant impact is greater at these levels.

Similarly to a laboratory result based on only one species, a single biochemical marker measured in an organism would have limited ecological significance. Thus, a selected suite of biological markers of exposure and of effects based on the type of expected contamination should be used in order to provide an integrative view of a studied situation. Based on the variability of biological markers as observed on a limited number of individual collected in the field, an appropriate number of individuals to be collected as representative of a population in a larger field study may be calculated using a power analysis - this number often varies between 15 and 30 individuals in freshwater environments (Hodson et al. 1993), but could be slightly higher for open marine environments. Although biomarkers can be applied in the laboratory, the following discussion relating to biological markers of exposure and of effects is oriented towards field investigations.

Mixed Function Oxygenase Enzymes

One of the most applied biochemical marker of exposure to contamination is the induction of the mixed function oxygenase (MFO) enzymes. This detoxification system found in most plants and animals has evolved because organisms have had to deal with foreign compounds since life began. MFOs are present with relatively low activity in all normal animals; however, if the organism is stressed by exposure to various bioavailable xenobiotics, MFO activity increases significantly to enhance the degradation and clearance of the offending chemicals (Dodson, 1991). Because certain MFO enzymes are also responsible for metabolism of multi-ringed corticosteroids (e.g. testosterone, estrogen), it is believed that elevation of MFO activity following exposure to xenobiotics may reduce levels of endogenous hormones resulting in altered fecundity and reproductive success. As such, the induction of MFO activity has been proposed as the basis for diagnosing exposure to several large classes of multi-ringed compounds and offers the potential of linking a biochemical indicator of chemical stress to a specific population response (Dodson, 1991). Although correlative relationships between low steroid hormones and elevated MFO activity have been observed in fish (Gagnon et al. 1995), no direct cause-and-effect association between these two factors had been established to date.

In fish, highest MFO activity is found in the liver where one atom of molecular oxygen (O_2) is incorporated into an hydrophobic multi-ringed contaminants (Di Guilio et al. 1995). The induction of hepatic MFO enzymes and excretion of metabolites via the kidneys or the bile may prevent accumulation of foreign compounds in fish (Holdway et al. 1995). Studies have demonstrated that hepatic MFO activity is largely increased following exposure of fish to petroleum compounds (George et al. 1995; Vandermeulen et al. 1994; Gagnon and Holdway *in press*). Because of the extensive metabolism and excretion of petroleum compounds by fish, direct measurement of tissue concentrations generally does not provide a useful indicator of exposure of fish to petroleum compounds (Collier et al. 1996). For example, George et al. (1995) showed that there was no significant petroleum compounds in muscle tissue from fish sampled three months after an oil spill incident, but that oil components had in fact been assimilated by the fish and had induced increases in the measured MFO levels. The induction of MFO activity, as measured by one specific enzymes ethoxyresorufin-*O*-deethylase (EROD), can be complemented by additional selected markers of exposure to provide evidence of bioavailability, uptake and metabolism of petroleum hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs). Assessing exposure of fish to PAHs is of special interest because of the general recognition that this class of petroleum hydrocarbons has high potential for exerting toxicity. An additional concern is that some electrophilic intermediates of high-molecular-weight PAHs resulting from MFO induction can form covalent adducts with nuclear DNA (Collier et al. 1996).

Some Australian crude oils such as Bass Strait crude oils are classified as light crude oils, with aromatic hydrocarbons accounting for up to 45% of the total hydrocarbons (Volkman et al. 1994). As aromatic hydrocarbons are relatively soluble in water, it is expected that the potential toxicity of light Australian oils to adversely affect aquatic organisms is higher than for heavier, less water-soluble oils. The high potential of these oils to induce MFO detoxification enzymes in fish has been demonstrated by exposure of Atlantic salmon (*Salmo salar*) to a low-level water accommodated fraction derived from an oil-water mixture using Bass Strait crude oil (Fig.1B). The comparative potential of other Australian crude oils to induce MFOs in fish remains to be demonstrated.

Because MFO induction is a reliable indicator of exposure to multi-ringed contaminants in fish, MFO activity measurements have been included in the North Sea Monitoring Master Plan as well as in the Canadian Federal Fisheries Act 1991 (Hodson et al. 1991). There are, however, important limitations to the use of MFOs in field monitoring. The first constraint relates to the fact that no deleterious biological effect has been directly related to the MFO induction. Although several studies correlate MFO induction to population level effects, causal relationships yet remain to be clarified. In this regard, induction of MFO is regarded as a marker of exposure to certain classes of contaminants rather than a marker of biologically significant effects. The second limitation refers to variable exposure levels under field conditions, especially for non-sedentary fish species, making difficult assessment of the relationship between exposure levels and MFO induction (Holdway et al. 1995). Effective field studies can minimize these limitations by selecting a suite of complementary biochemical markers of exposure measured in a well-known bioindicator species.

SORBITOL DEHYDROGENASE

Sorbitol dehydrogenase (SDH), an enzyme involved in the interconversion of fructose and sorbitol, is localized primarily in the liver. Normally very little, if any, is present in the bloodstream; the appearance of SDH activity in the serum is then indicative of hepatocellular injury related to exposure to xenobiotics. Thus, this biochemical marker indicates a biologically significant measure of adverse effects. SDH activity is not altered by conditions often affecting MFO induction such as reproductive activity. In a monitoring program, serum SDH activity can be used in conjunction with MFOs, and can explain discrepancies in measured MFO levels due to cellular liver damages (Dixon et al. 1987). Fish livers experiencing cellular injuries due to xenobiotic exposure are then less capable of MFO induction as non-injured livers are. Hepatocellular injury represents a confounding factor in interpreting MFO induction and supports the essential use of a marker of liver damage such as SDH activity when using MFO induction as a biochemical marker of xenobiotic exposure. In fish collected from Port Phillip Bay, for example, elevated serum SDH activity was associated to reduced EROD induction in fish exposed to high contaminant levels (Holdway et al. 1994).

BILE METABOLITES

As previously mentioned, direct measurement of tissue concentrations of petroleum compounds generally does not provide a useful indicator of exposure of fish to petroleum hydrocarbons specifically because of the high rate of metabolism and depuration of hydrocarbons by many species of fish (Collier et al. 1996). Petroleum hydrocarbons as PAHs are metabolized by the MFO system to more hydrophilic products like phenols, dihydrodiols, quinones and epoxides; these polar metabolites are then subjected to a conjugaison with glutathione, sulfate or glucorinic acid prior being excreted via the bile (Van der Oost et al. 1994). Other excretion routes are possible, but minimal excretion of petroleum compounds via the kidneys is expected as the major route of excretion of high molecular weight metabolites has been shown to be biliary (Klaasen and Rozman 1991). The presence of metabolites of xenobiotics in the bile indicates that the compounds are bioavailable, and that absorption, metabolism and excretion of the contaminants has occurred. Although adverse impacts at the organ/organism levels may derive from biologically active metabolites, the measurement of bile metabolites does not represent a measure of effects but rather a measure of exposure to petroleum compounds.

Although bile metabolites can be detected as rapidly as MFO induction (approximately 2 days after exposure), the persistence of metabolites in the bile permits the use of the marker as a sensitive indicator of exposure for long term assessments. For example, bile metabolites have been detected in fish collected at 640 km of an oil spill site, one year after the event (Collier et al. 1996). Traditional methods of estimating bile metabolite content used time-consuming techniques and sophisticated equipment. However, recently developed methods allow simple and rapid detection of bile metabolites classified into naphtalene or benzo(a)pyrene-types of metabolites by fixed fluorescence (Lin et al. 1996). Accumulation of metabolites in the bile of Atlantic salmon have been tested with Bass Strait crude oil and proved to be a reliable indicator of uptake and depuration of petroleum compounds by this fish species (Fig. 2). Similar results have yet to be generated using native Australian species; this technique has been applied to numerous North American fish species (Lin et al. 1996) and its practice in Australia is imminent. Simplicity of this

method promises determination of biliary PAH metabolites to become a routine measurement endpoint for environmental assessments using fish.

METABOLIC ENZYMES

In animals, available energy is divided between various activities namely growth, reproduction and basal metabolism. Perturbations in metabolism is common in fish inhabiting contaminated environments, as observed by higher lipid accumulation (Sandström et al. 1988) and faster growth rates (Gagnon et al. 1995) in fish chronically exposed to contaminants. Perturbations of the carbohydrate metabolism originates from biochemical dysfunctions at the subcellular level, and assessment of functional alterations of metabolism can provide insights on the mode of action of a xenobiotic as well as on the ecological significance of chronic exposure to contaminants. It has been suggested that anionic uncouplers as petroleum hydrocarbons may alter the cellular integrity by partitioning into the phospholipids of mitochondrial membranes and dissipating the energy gradient by transporting protons through this membrane (Shannon et al. 1991).

Traditionally used in investigations of physiological character, aerobic and anaerobic metabolic enzymes had proven useful in understanding observed disturbances of energetic metabolism. Aerobic capacity of an organ can be estimated by the activity of citrate synthase (CS), the first enzyme of the Krebs cycle located within the mitochondria, and by the activity of cytochrome C oxidase (CCO), the terminal enzyme of the electron transport system found in the inner membrane of the mitochondria. By selecting these two representative enzymes located at the beginning and at the end of the aerobic respiratory cycle, one can integrate the entire aerobic process and capture disruption occurring at any site within the cycle. In the event of an impairment in the aerobic capacity, it is expected that compensation mechanisms would counterbalance the lost of aerobic capacity by increasing the anaerobic capacity of a tissue so that the total metabolic capacity of a tissue remains stable. Therefore, determination of aerobic enzymes has to be complemented by the measurement of the anaerobic enzyme lactate dehydrogenase (LDH), the terminal enzyme of the anaerobic glycolysis.

Fish exposed to petroleum compounds do demonstrate impairments in the carbohydrate metabolism as measured in the gills of Atlantic salmon exposed to water accommodated fraction of Bass Strait crude oil (Gagnon and Holdway, submitted). Measurement of these enzymes in crucial organs as gills can demonstrate a short-term initial impact of exposure; quantification in muscle, which comprise the largest part of the fish, also has great biological importance as fish depend heavily on the capacity of their muscle for motility. Perturbation in metabolic enzymes represent a biologically significant effect especially in chronic exposures, as biological implications of metabolism include impaired growth and locomotory activity, and reduced survival probability in wild fish (Pride 1977).

STRESS PROTEINS

The use of stress protein in environmental toxicology is at its infancy, but enough is known from studies of physiology to warrant the induction of these proteins as a general, long-term marker of exposure to environmental stressors. Stress proteins are synthesized under normal conditions; exposure to adverse conditions, even natural, may results in cellular proteins denaturation through the weakening of polar bonds and exposure of hydrophobic groups, resulting in misfolding and cellular protein aggregation. The role of stress proteins is to repair denatured proteins and protect cellular proteins from environmentally induced damages (Sanders 1993).

The ubiquitous stress protein SP-70 is the most highly conserved amongst animals. The induction, responsive to a wide variety of stressors including aquatic contaminants, is slow (>24h exposure is necessary) and does not appear to be influenced by handling of the organism (Vijayan et al. 1997). Because the extend of the stress response is closely linked to protein damage at the tissue level, stress protein induction appear to be tissue specific. Thus, an hepathotoxic agent would induce stress proteins mainly in the liver, while a non-organ specific toxic compound may induce stress proteins in the entire organism. Data from recent studies suggest that the accumulation of SP-70 reflects the integrated stress load on the aquatic organism regardless of the type and number of stressors involved, and that accumulation of high levels of SP-70 is persistent upon continuous exposure to stressors such as elevated temperatures, or contaminants. Although induction of stress protein SP-70 by Australian crude oils have not been demonstrated in fish, preliminary results indicate that these crude oils are strong stress protein inducers in the invertebrates *Mytilus edulis* and *Octopus pallidus* (S. Long, RMIT, Melbourne, pers. comm.).

Any environmental conditions that increases protein denaturation is expected to induce the classical stress protein response; for example, reproductive activity, moulting, or resorption of gametes are associated with elevated levels of SP-70 (Sanders 1993). Stress proteins are therefore a relatively non-specific indicator of cellular stress. As for several other biological markers applied in field studies, careful experimental design, appropriate reference organisms and good knowledge of the bioindicator's biology will minimize variability in the measured parameter.

DNA ADDUCTS

During the process of metabolism and excretion, electrophilic intermediates of high molecular weight PAHs can form covalent *adducts* (*add*-ition prod-*ucts*) with desoxyrobonucleic acid (DNA). PAHs metabolites with four or more condensed benzene rings are often mutagenic and/or carcinogenic, and the prevalence of neoplasia in fish has been associated with elevated PAH levels in the environment (Lin et al. 1996). It appears that a higher activity of the MFO system increases PAH biotransformation in fish inhabiting polluted sites, which in turn decreases the PAH body burden but favors formation of DNA adducts (Van der Oost et al. 1994). Thus, quantification of DNA adducts in fish represent a biologically significant effect related to chronic exposure of fish to xenobiotics. In field studies, this marker can be used during continuous long-term exposure of organisms to xenobiotics or to evaluate recovery of a site after an event.

Methods applied to the detection of DNA adducts are extremely sensitive, detecting one alteration in 10^9-10^{10} bases on the DNA molecule (Reichert and French 1994). The non-specific nature of the technique allows for the detection of a wide range of bulky, hydrophobic compounds bound to DNA, without precise identification of the specific metabolite responsible for the adduct formation. However, because fish are usually exposed to poorly characterized mixtures of contaminants, the ability to detect DNA adducts of unknown structure is a key advantage in field studies.

CONCLUSIONS

The purpose of environmental health assessments is to identify problems and propose remedial procedures in order to conserve or restore environmental quality in a cost-effective manner. However, comprehensive chemical characterization of the environment, and interactive effects of xenobiotics in areas of mixed pollution are enormous tasks practically impossible to achieve. Adverse effects at the individual organism level will at some stage result in impacts at the population, community and ecosystem level of biological organization (McCarthy and Shugart, 1990). Through determination of a suite of biological markers of exposure and of effects in integrator organisms, impacts at the individual level may be used as an early warning indicator of ecosystem deterioration.

The responses observed in field studies effectively integrate the direct and indirect effects of chemical exposure and environmental conditions. Numerous examples of field studies demonstrated the importance of selecting not only one but a suite of biological markers of exposure and of effect. Results from a suite of biological markers can often provide a clearer picture of the degree and timing of contaminant exposure, leading to a better understanding of related biological effects and their ecological significance. A array of short- and long-term biological markers is now available to assess the exposure and effects related to contamination of the environment; selective combination of these markers can effectively provide concerned environmental managers with an assessment of the induced environmental impacts. Results of a carefully planned sampling design considering ecoepidemiological concepts will strengthen evidences relating responses at the individual level and effects at the population and community levels.

REFERENCES

Collier T.K., C.A Krone, M.M.Krahn, J.E. Stein, S.-L. Chan, and U. Varanasi. 1996. Petroleum exposure and associated biochemical effects in subtidal fish after the *Exxon Valdez* oil spill. American Fisheries Society Symposium 18:671-683.

Di Guilio R.T., W.H. Benson, B.M. Sanders and P.A. Van Veld. 1995. Biochemical mechanisms: metabolism, adaptation, and toxicity. P. 523-561 In. G.M. Rand (Ed) Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment. Second Edition, Taylor and Francis, Washington D.C.

Dixon D.G., P.V. Hodson and K.L.E. Kaiser. 1987. Serum sorbitol dehydrogenase activity as an indicator of chemically induced liver damage in rainbow trout. Environ. Toxicol. Chem. 6:685-696.

Dodson J.J. 1991. Environmental health assessment at the population level: xenobiotic induction of P-450 mixed-function oxidase and reproductive failure in fishes. P. 31-87. In: Inland Aquatic Environmental Stress Monitoring, BIOTROP Spec. Publ. No.43.

Evans L., J.F. Spickett, J.R. Bidwell, R.J. Rippingale, and H.L. Brown. 1994. Application of ecotoxicology to environmental management in the Australian offshore oil and gas industry. APEA Journal 34:809-820.

Gagnon M.M., J.J. Dodson and P.V. Hodson. 1994. Ability of BKME (bleached kraft mill effluent) exposed white suckers (*Catostomus commersoni*) to synthesize steroid hormones. Comp. Biochem. Physiol. 107C:265-273.

Gagnon M.M., D. Bussieres, J.J. Dodson and P.V. Hodson. 1995. White sucker (*Catostomus commersoni*) growth and sexual maturation in pulp-mill-contaminated and reference rivers. Environ. Toxicol. Chem. 14:317-327.

Gagnon M.M. and D.A. Holdway. *In press.* MFO induction in Atlantic salmon *(Salmo salar)* during and after exposure to Bass Strait crude oil. *In press* in Aust. J. Ecotox.

Gagnon M.M. and D.A. Holdway. Submitted. Metabolic enzymes in fish gills as biomarker of exposure to petroleum hydrocarbons. Submitted in Ecotox. Environ. Saf.

George S.G., J. Write and J. Conroy. 1995. Temporal studies of the impact of the Braer oilspill on inshore feral fish from Shetland, Scotland. Arch. Environ. Contam. Toxicol. 29:530-534.

Hodson P.V., J.J. Dodson, D. Bussières and M.M. Gagnon, 1993. Fish Population monitoring - How many fish are enough? Proceedings of the 20th Aquatic Toxicity Workshop, October 21, 1993, Canada, pp.43-47.

Hodson, P.V., P.J. Kloepper-Sams, K.R. Munkittrick, W.L. Lockhart, D.A. Metner, P.L. Luxon, I.R. Smith, M.M. Gagnon, M. Servos and J.F. Payne, 1991. Protocols for measuring mixed function oxygenases of fish livers. Can. Tech. Rep. Fish. Aquat. Sci. No. 1829, 51 p.

Holdway D.A., S.E. Brennan and J.T. Ahokas. 1995. Short review of selected fish biomarkers of xenobiotic exposure with an example using fish hepatic mixed-function oxidase. Aust. J. Ecol. 20:34-44.

Holdway D.A., S.E. Brennan and J.T. Ahokas. 1995. Use of hepatic MFO and blood enzyme biomarkers in sand flathead (*Platycephalus bassensis*) as indicators of pollution in Port Phillip Bay, Australia. Mar. Pollut. Bull. 26:683-695

Klassen C.D. an K. Rozman. 1991. Absorption, distribution and excretion of toxicants. P.50-87. In: Toxicology: the basic science of poisons, 4th edn, M.O. Amdur, J. Doull and C.D. Klaasen (Eds). Plenum Press, New York.

Lin E.L.C., S.M. Cormier and J.A. Torsella. 1996. Fish biliary polycyclic aromatic hydrocarbon metabolites estimated by fixed-wavelength fluorescence: comparison with HPLC-fluorescent detection. Ecotoxicol. Environ. Saf. 35:16-23.

McCarthy J.F. and L.R. Shugart. 1990. Biological markers of environmental contamination. P. 3-14. IN: Biomarkers of environmental contamination. J.F. McCarthy and L.R. Shugart (Eds), Lewis Publishers, CRC Press Inc., Florida.

Niimi A.J. 1990. Review of biochemical methods and other indicators to assess fish health in aquatic ecosystems containing toxic chemicals. J. Great Lakes Res. 16:529-541.

Pride I.G. 1977. Natural selection for energetic efficiency and their relationship between activity level and mortality. Nature. 267:610-611.

Reichert W L. and B. French. 1994. The ^{32}P-postlabelling protocols for assaying levels of hydrophobic DNA adducts in fish. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-14, 89 p.

Sanders B. 1993. Stress proteins in aquatic organisms: an environmental perspective. Crit. Rev. Toxicol. 23:49-75.

Sandström O., E. Neuman and P. Karås. 1988. Effects of bleached pulp mill effluent on growth and gonad function in Baltic coastal fish. Wat. Sci. Technol. 20:107-118.

Smith B.J. and M.M. Gagnon. *In press*. MFO induction of three Australian fish species. *In press*, Environ. Toxicol. Water Qual.

Shannon R.D., G.D. Boardman, A.M. Dietrich and D.R. Bevan. 1991. Mitochondrial response to chlorophenols as short-term toxicity assay. Environ. Toxicol. Chem. 10:57-66.

Van der Oost R., F.J. Van Schooten, F. Ariese, H. Heida, K. Satumalay and N.P.E. Vanmeulen. 1994. Bioaccumulation, biotransformation and DNA binding of PAHs in feral ell (*Anguilla anguilla*) exposed to polluted sediments: a field survey. Environ. Toxicol. Chem. 13:859-870.

Vandermeulen J.H., V. Vignier and D. Mossman. 1994. Toxicology of Hibernia crude oil in parr and smolt of Atlantic salmon (*Salmo salar*). Proceedings of the 17th AMOP Technical Seminar, Vol. 2: 1287-1301.

Vijayan M.M., C. Pereira, R.B. Forsyth, C.J. Kennedy and J.K. Iwama. 1997. Handling stress does not affect the expression of hepatic heat shock protein 70 and conjugation enzymes in rainbow trout treated with ß-naphtoflavone. Life Sciences, 61:117-127.

Volkman J.K., G.J. Miller, A.T. Revill and D.W. Connell. 1994. Oil Spills, P.509-695. In: Environmental implications of offshore oil and gas development in Australia - the findings of an independent scientific review. J.M. Swan, J.M. Neff and P.C. Young (Eds). Australian petroleum Exploration Association, Sydney.

Figure 1. (A) MFO induction, as measured by EROD activity, in southern sand flathead non-injected (N = 10), injected with corn oil only (N = 13) or i.p. Injected with 100 ug/kg PCB isomer # 126 diluted in corn oil (N = 9). A 20-times EROD induction indicates that this species can be an excellent bioindicator of environmental contamination by hydrophobic xenobiotics as pcbs and pahs (adapted from Smith and Gagnon, in press). (B) EROD induction in Atlantic salmon exposed for the first 4 days to diluted (0.025% of a 1:99 oil:seawater mixture) Bass Strait water accommodated fraction followed by 57 days in clean seawater. N = 16 salmon/day (adapted from Gagnon and Holdway, in press).

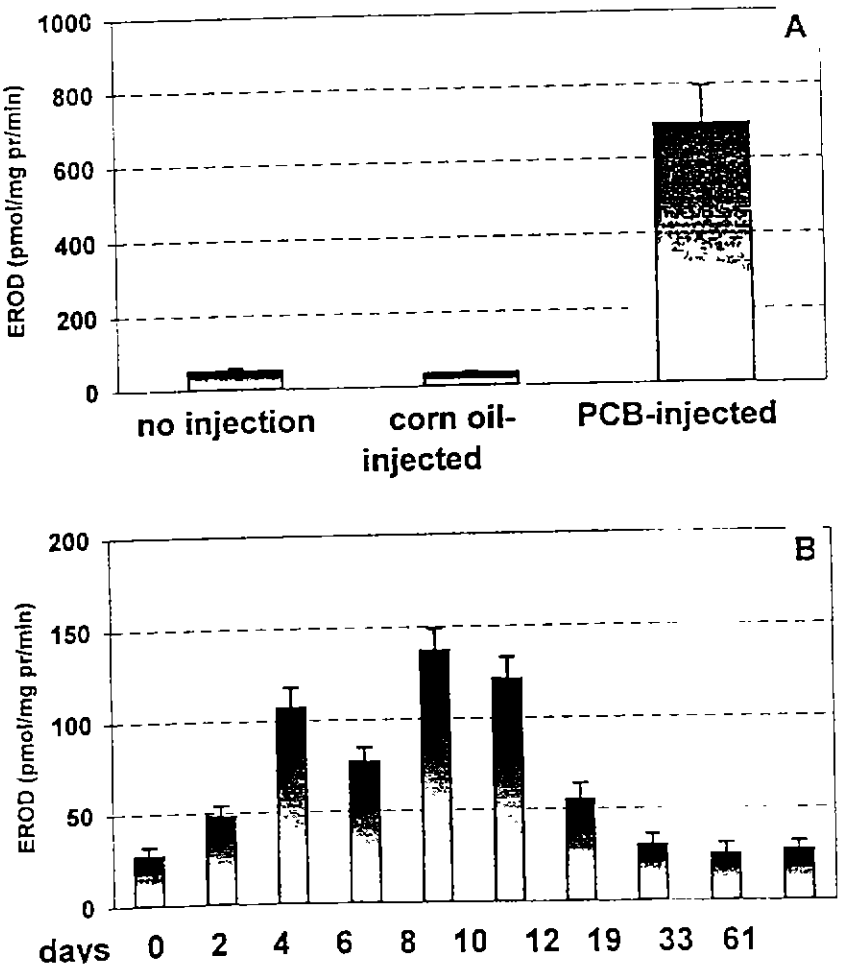

Figure 2. Accumulation of naphtalene-type metabolites in the bile of Atlantic salmon exposed to 1% water accommodated fraction of Bass Strait crude oil (1:9 oil:seawater) for 6 days followed by a depuration period. N = 8 salmon/day (Gagnon and Holdway, in prep.)