Ecotoxicology, 15, 51–59, 2006

DOI: 10.1007/s10646-005-0041-5

Evaluation of the Gill Filament-Based EROD Assay in African Sharptooth Catfish (*Clarias gariepinus*) as a Monitoring Tool for Waterborne PAH-Type Contaminants

ROBINSON MDEGELA,[1,*] JAN MYBURGH,[2] DACIA CORREIA,[3] MARTE BRAATHEN,[4] FRANCIS EJOBI,[5] CHRISTO BOTHA,[2] MORTEN SANDVIK[6] AND JANNECHE UTNE SKAARE[4,6]

[1]*Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania*

[2]*Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa*

[3]*Veterinary Faculty, University of Eduardo Mondlane, P.O. Box 257, Maputo, Mozambique*

[4]*Department of Pharmacology, Microbiology and Food Hygiene, Norwegian School of Veterinary Science, Dep., 0033, P.O. Box 8156, Oslo, Norway*

[5]*Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda*

[6]*Norwegian Veterinary Institute, Dep., 0033, P.O. Box 8146, Oslo, Norway*

Accepted 15 May 2005/Published online 1 December 2005

Abstract. The ability of African sharptooth catfish (*Clarias gariepinus*) in inducing cytochrome P-450 class 1A (CYP1A) and glutathione *S*-transferase (GST) biomarkers was determined in liver and gill filaments after 4 days of waterborne exposure to the polycyclic aromatic hydrocarbon, benzo[a]pyrene (B[a]P). Male ($n = 6$) and female ($n = 6$) fish were exposed to B[a]P added to the water (30 μg/l) corresponding to 5 mg/kg total body weight. Five female and six male fish, exposed to acetone alone added to the water served as controls. The 7-ethoxyresurufin-*O*-deethylase (EROD) activity was measured in hepatic microsomes and gill filaments. In addition, GST activity was determined in the hepatic cytosolic fraction and fluorescent aromatic compounds (FACs) in bile and biliverdin. Benzo[a]pyrene strongly induced EROD activities in gill filaments of both sex. Levels of FACs per ml of bile were 17-fold higher in exposed fish compared to the controls. Correlations between induction of EROD activities in gill filaments and liver and between induction of EROD activities in gill filaments and levels of biliary FACs metabolites were strong. GST activities in the hepatic cytosolic fraction were similar amongst the treated and control groups. This is the first report on studies determining EROD activities in gill filaments and hepatic tissue, FACs in bile and GST in hepatic tissues of *C. gariepinus* after waterborne exposure to B[a]P. The findings suggest that the gill filament-based EROD assay in *C. gariepinus* can be used to monitor the pollution of AhR agonists in aquatic ecosystems in eastern and southern African countries.

Keywords: benzo[a]pyrene; *Clarias gariepinus*; African catfish; gill and liver EROD assay; biomarkers

*To whom correspondence should be addressed:
Tel.: +255-23-260-45-42; Fax: +255-23-260-46-47;
E-mail: rmdegela@yahoo.com

Introduction

Induction of CYP1A in fish has been widely used as a sensitive and convenient early warning signal of exposure to aryl hydrocarbon (AhR) agonists such as polychlorinated biphenyls, dioxins, furans, organochlorine pesticides and polycyclic aromatic hydrocarbons (PAHs) (Payne et al., 1987; Goksøyr and Förlin, 1992; Beyer et al., 1996; Jönsson, 2003). A frequently used method for analysis of CYP1A induction is measuring the catalysed O-deethylation of ethoxyresorufin (EROD), which is used as a substrate. In fish, EROD induction is usually determined in hepatic microsomes. Pollutants absorbed via the gills may be metabolised before reaching the liver, thus hepatic EROD activity may not be the most sensitive organ to reflect the presence of CYP1A inducing agents in water. As in many other organs, CYP1A is also induced in fish gills following exposure to PAHs, and a sensitive method to determine EROD activities in gill filaments has recently been described by Jönsson et al. (2002). The assay is handy, robust and relatively cheap. Whereas hepatic EROD assay requires several steps of tissue preparation, this method utilises intact tissue. In addition to saving time, this method does not require expensive equipments such as ultra centrifuges and ultra deep freezers. Thus, this method constitutes a new measure to reveal waterborne CYP1A inducers and has proven to be a useful biomarker in several cold-water species, but is virtually unproven in tropical species. *Clarias gariepinus*, a bottom feeder, fresh water fish native to Africa, is highly tolerant to extreme climatic conditions. This fish species is widely distributed in many eastern and southern African countries. Although some studies related to environmental pollution have been conducted in *C. gariepinus* (Timmers et al., 1988; Theron et al., 1991; Lien et al., 1997; Nguyen et al., 1999; Datta and Kaviraj, 2003; Mbongwe et al., 2003; Viljoen et al., 2003), limited information on the potential use of this fish species in environmental pollution monitoring programmes is available.

To date, little or no information is available on the occurrence and sources of PAHs in African countries. Overall levels of PAHs are in general well below levels found in urbanised and industrialised locations around the world (Jaward et al., 2004). However, sediments and effluents from local point sources have shown to be highly contaminated with PAHs and other potent inducers of CYP1A (Gadagbui and Goksøyr, 1996; Olajire et al., 2005).

Another biomarker for exposure to PAHs is measurement of biliary fluorescent aromatic compounds (FACs). A strong correlation between fish exposure to PAHs and biliary FACs has been demonstrated in previous studies (Krahn et al., 1986; Collier and Varanasi, 1991; Beyer et al., 1996, 1997). Such studies used a HPLC method with fluorescence detection (HPLC/F) that was developed by Krahn et al. (1980) as a rapid screening method for estimating relative amount of biliary PAH metabolites in fish without complete separation or identification of individual metabolites.

Metabolised PAHs such as B[a]P are one of the examples of biotransformed electrophilic xenobiotics. A common mechanism for protection of an individual against biotransformed electrophilic xenobiotics is conjugation with glutathione (GSH) in phase II detoxification reactions, (Mitchell et al., 2000). Although the induction of enzymes such as glutathione *S*-transferases (GSTs) which are involved in phase II detoxification reactions have been found to be inconsistent in different fish species, yet the determination of GSTs activities is often considered as a potential auxiliary biomarker for exposure of fish to organic compounds (Gallagher et al., 2001). However, there is limited information regarding the induction of GSTs in *C. gariepinus* following waterborne exposure to PAHs.

The main objective of this study was to evaluate the gill filament EROD assay under tropical climatic conditions using *C. gariepinus* as the model species, and comparing CYP1A induction in liver and gills following waterborne exposure to B[a]P. Furthermore, accumulation of FACs metabolites in bile and GST enzyme activities in hepatic cytosolic fractions were measured.

Materials and methods

Chemicals

7-ethoxyresorufin, resorufin, NADPH, glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB) and

bovine serum albumin were obtained from Sigma – Aldrich (St Louis, Mo, USA). A protein determination kit was obtained from Bio-Rad Laboratories GmbH (Munchen, Germany) and B[a]P from Birkenhead, Merseyside, UK. All other chemicals and solvents used were of analytical grade.

Fish

Female ($n=11$) and male ($n=12$) African sharptooth catfish (*C. gariepinus*) with mean body weight of 340 ± 90 and 450 ± 130 g, respectively, were used in the experiment. Fish were caught at local dams and acclimatised for 2 weeks in a large tank before they were transferred to smaller glass tanks (120–140 l). The water was aerated with air pumps and the photoperiod was set at 12 h light and 12 h dark cycle. Water temperature was 19.5 ± 0.1 °C with 7 mg/l dissolved oxygen. The pH ranged from 7.7 to 7.9 and no ammonia and nitrate build-up was detected. The fish were not fed during the acclimatisation and challenge periods.

Treatment

Male ($n=6$) and female ($n=6$) fish were exposed to B[a]P, dissolved in acetone before being added to the water, at a dose of 30 μg/l corresponding to 5 mg/kg total body weight. Five female and six male fish were used as controls and were exposed only to acetone added to the water. Following the exposure period of 4 days the fish were sacrificed by decapitation and pithing. The gills were removed and used for analysis of CYP1A enzyme activities. The liver samples were collected and stored in liquid nitrogen for analysis of CYP1A and GST enzyme activities.

Preparation and incubation of microsomes

Liver microsomal fractions were prepared and EROD activities determined according to the methods described by Beyer et al. (1996). Briefly, individual liver samples were weighed and homogenised in 0.1 M Na-phosphate buffer containing 0.15 M KCl (pH 8.0) in a volume four times the liver weight using a Potter-Elvehjem glass – Teflon homogeniser and centrifuged at $12{,}000 \times g$ for 20 min at 4 °C. The supernatant (S12 fraction) was ultracentrifuged at $100{,}000 \times g$ for 60 min at 4 °C. The microsome pellet was resuspended and homogenised in 0.1 M Na-phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM dithiothreitol and 20% glycerol. Microsomal and cytosolic fractions were stored at −80 °C until use. The protein content was determined by the method described by Bradford (1976), using lyophilised bovine serum albumin as standard.

The total incubation volume for sample microsomes was 1500 μl and it included 5 μl of 0.5 mM ethoxyresorufin, 40 μl of 10 mM NADPH, 20 μl sample microsomal fraction and 1435 μl of 0.1 M phosphate buffer (pH 7.4). The blank consisted of 5 μl of 0.5 ethoxy-resorufin, 40 μl of 10 mM NADPH and 1455 μl of 0.1 M phosphate buffer pH 7.4. The reaction started by adding the cofactor (NADPH) and after 30 min the reaction was stopped by adding 1.5 ml ice-cold methanol. After stopping the reaction, tubes were centrifuged at $3100 \times g$ for 30 min and the supernatant transferred to the HPLC vials for further analysis as described below.

Preparation and incubation of gill filaments

Gill filaments were prepared and EROD activities were determined according to the methods described by McCormick and Bern (1989) and Jönsson (2003). The gill arches were excised and placed in ice-cold HEPES-Cortland (HC) buffer (pH 7.4). While immersed in HC buffer, the tips of the filaments were immediately cut into pieces of approximately 2 mm long. From each fish, 10 gill filament tips of 2 mm long were carefully selected and transferred with a Pasteur pipette to wells on a 12 well tissue culture plate containing HC buffer. There were two replicates for each incubation time. The HC buffer was replaced with 0.5 ml of reaction buffer (HC buffer supplemented with 10^{-6} M ethoxy-resorufin and 10^{-5} M dicoumarol). Following 2 min of pre-incubation at room temperature, the buffer was replaced with 0.7 ml of fresh reaction buffer. After 10, 30 and 60 min, 0.2 ml aliquots were transferred from each well to a HPLC vial. The EROD activity was determined and expressed as picomoles of resorufin per minute per filament tip.

EROD analysis

The dealkylation of 7-ethoxyresorufin is usually monitored directly either with spectrophotometric or fluorometric assays. Different protein concentrations in the assay mixture is, however, a problem in terms of quenching of the signal. The degree of quenching is corrected for by adding a known amount of resorufin. This method avoids the problem by precipitating protein by adding methanol to the incubation mixture. Following centrifugation, the supernatant is transferred to HPLC vials.

Resorufin was identified and the concentration calculated by comparison to retention times and response of known standards using a HPLC equipped with a Perkin Elmer LS 4 fluorescence detector at an excitation wavelength of 535 nm and an emission wavelength of 585 nm. For analysis 5 µl aliquots of the centrifuged incubation mixtures or the reaction buffer were injected onto a Symmetry C18, 150 × 3.9 mm i.d., 5 µm, column (Waters, Milford USA), with a guard column packed with the same material. Resorufin was eluted at a flow rate of 1 ml/min with a mobile phase containing 60% acetonitrile:40% Na-phosphate buffer (0.1 M; pH 7.4). The linearity of resorufin standards ranged from 0.02 to 4.4 pmol and the limit of quantification was 0.005 pmol resorufin.

HPLC analysis of water and bile samples for B[a]P and FACs

Aliquots of 5 µl bile samples were diluted 1:2000 with acetonitrile:water (90:10) and analysed by HPLC with fluorescence detector according to Krahn et al. (1987). Water samples that were collected daily (Table 1) were analysed according to Clements et al. (1994). Thirty millilitres of water was extracted with bonded reverse-phase cartridges (Varian Bond Elut LRC^{TM}-C18). Cartridges were air-dried and B[a]P was eluted with 10 ml of ethyl acetate. The eluate was evaporated under nitrogen and the residue dissolved in 200 µl acetonitrile:water (9:1) prior to HPLC analysis. Concentrations of B[a]P-type fluorescent compounds (FACs) in water and bile samples were determined. Twenty microlitres of sample or standard was eluted at a flow rate of 1.5 ml/min with a mobile phase containing 90% acetonitrile and 10% water. The areas of fluorescent peaks at excitation/emission 380/430 nm were summed and converted to B[a]P equivalents, based on areas of known B[a]P standards.

Table 1. Mean concentrations (ng/l) of BaP in water during exposure of *C. gariepinus*

Day	*n*	Mean	SEM
1	4	252	244
2	2	85	48
3	4	117	85

Biliverdin content of bile was estimated by the method of Grossbard et al. (1987), which involves measuring the absorbance of a biological fluid at 660 nm and determining the concentration of biliverdin based on its extinction coefficient of 10,800/mM/cm.

Determination of GST

The hepatic GST activity was measured spectrophotometrically (Beckman UV–visible Spectrophotometer) in cytosolic fractions according to Habig et al. (1974) using CDNB and GSH at pH of 7.4. The assay was performed by transferring a 3 ml reaction mixture that contained 2 ml of 0.8 mM CDNB dissolved in 0.1 M Na-phosphate buffer (pH 7.4), 990 µl GSH and 10 µl of sample into a 3 ml cuvette. Increases in absorbance at 340 nm were recorded for 40 s at intervals of 10 s. The GST activities were quantified using an extinction coefficient for glutathione of 9.6/mM/cm.

Statistical methods

Data were analysed using the JMP® Software for Statistical Visualisation (SAS Institute Inc.). Two factor ANOVA was used to determine differences between groups with treatment as a variable and enzyme activity as a response at critical probability of $p < 0.05$. In cases where a significant difference was indicated, Dunnett's test for multiple comparisons of means was used to test for differences between exposed and control groups. Spearman's Rank Correlation was used to test the direction and strength of the relationship between induction of EROD activities in gills and liver as well as

FACs metabolites in the bile of fish from different groups.

Results

The mean measured concentration of BaP in water in the exposure tanks was 252 ng/l at the start of the experiment and dropped to 117 ng/l at the end of the experiment (Table 1). The time course of resorufin formation in primary gill filaments of non-induced and B[a]P induced fish was linear (Fig. 1).

After waterborne exposure of *C. gariepinus* to B[a]P for 4 days, gill filaments demonstrated increased EROD activities upon treatment. Eleven and 17-fold increase in gill filament EROD activities were observed in exposed females and males compared to the corresponding unexposed females and males, respectively (Fig. 2). The mean CYP1A enzyme activities per minute per gill filament tip for treated females and males, and control females and males were 0.17 ± 0.03, 0.24 ± 0.03, 0.01 ± 0.01 and 0.02 ± 0.001 pmol/filament tip/min, respectively. Gill filament EROD activities were statistically significant between control and exposed groups ($p = 0.00005$), but not between unexposed males and females and exposed males and females. Correlations between EROD activities in gill filaments (at 30 min of gill incubations) and liver ($Rs = 0.57$, $p = 0.004$) and between EROD activities in gill filaments (at 30 min of gill incubations) and levels of biliary FACs metabolites ($Rs = 0.76$, $p = 0.001$) were strong. However, the correlation between EROD activities in liver and FACs metabolites in bile was weak ($Rs = 0.2$, $p = 0.49$).

After waterborne exposure of *C. gariepinus* to B[a]P for 4 days, liver tissues demonstrated increased mean measured EROD activities upon treatment (Fig. 3). Approximately 2-fold induction of hepatic EROD activities was observed in the exposed groups compared to the control groups. The mean hepatic EROD activity in unexposed females and males and in exposed females and males were 3.36 ± 0.49, 3.36 ± 0.57, 5.08 ± 1.02 and 5.32 ± 1.22 pmol/min/mg protein, respectively. However, no significant difference ($p = 0.29$) was observed between groups.

A 2-fold induction of GST was observed in hepatic cytosolic fractions in exposed females compared to the controls, but GST activity in exposed male fish was only slightly elevated (Fig. 4).

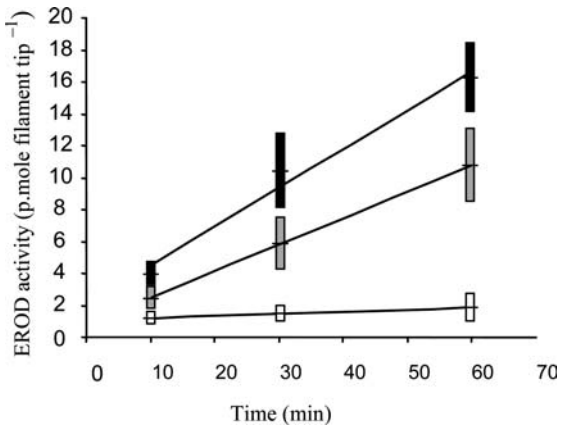

Figure 1. Time course of resorufin formation in primary gill filaments of non-induced and B[a]P-induced sharptooth catfish (*C. gariepinus*). The fish received B[a]P dissolved in acetone and added in the water (30 µg/l) or acetone only. The results are given as mean ± SE ($n = 5$ or 6). ⊟: Unexposed females; ⊟: exposed females; ■: exposed males.

The HPLC/F analysis revealed several unidentified peaks with fluorescence at excitation/emission 380/430 even from the start of the experiment (Fig. 5). The levels of FACs per ml bile were about 17-fold higher in treated females and males compared to control females. The mean levels of FACs for exposed females and males and control females

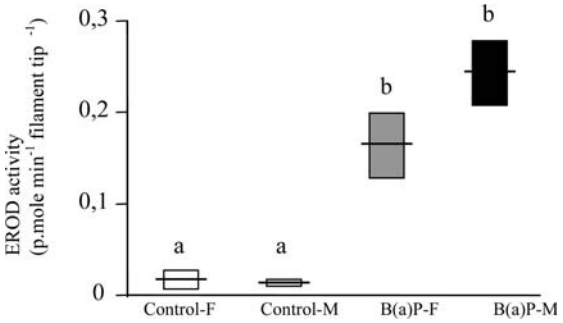

Figure 2. Induction of CYP1A enzyme activities in gill filaments of sharptooth catfish (*C. gariepinus*) after 4 days of waterborne exposure to B[a]P (30 µg/l). The results are given as mean ± SE ($n = 5$ or 6).⊟: Unexposed females; ⊟: unexposed males; ⊟: exposed females; ■: exposed males. Groups not connected by same letters are significantly different (Tukey–Kramer HSD).

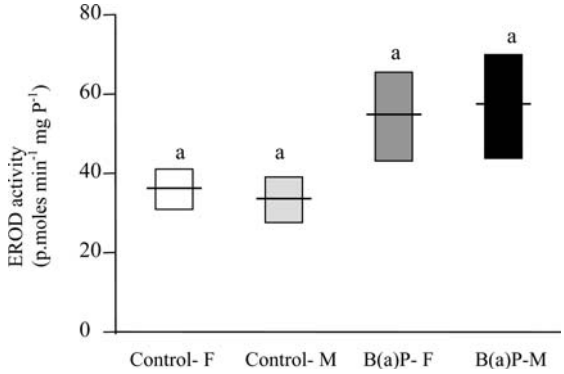

Figure 3. Induction of hepatic CYP1A enzyme activities in sharptooth catfish (*C. gariepinus*) after 4 days of waterborne exposure to B[a]P (30 μg/l). The results are given as mean ± SE ($n = 5$ or 6). ⊟: Unexposed females; ⊟: unexposed males; ■: exposed females; ■: exposed males. Groups not connected by same letters are significantly different (Tukey–Kramer HSD).

and males were 7.1 ± 0.8, 6.2 ± 1.3, 0.4 ± 0.1 and 0.41 ± 0.1 μg B[a]P equivalents/ml bile, respectively (Fig. 6). Means of FACs were significantly different between the exposed female and male fish compared to female and male fish in the control groups ($p = 0.0002$). Only trace amounts of the parent B[a]P were detected in the bile of exposed fish. When the levels of FACs were calculated per mg biliverdin, the same substantial increase was seen in the exposed fish compared to the controls. The mean levels of biliverdin for treated females and males, and control females and males were 1.5 ± 0.4, 1.4 ± 0.6, 1.3 ± 0.4 and 1.3 ± 0.5 μg B[a]P equivalents/mg biliverdin, respectively.

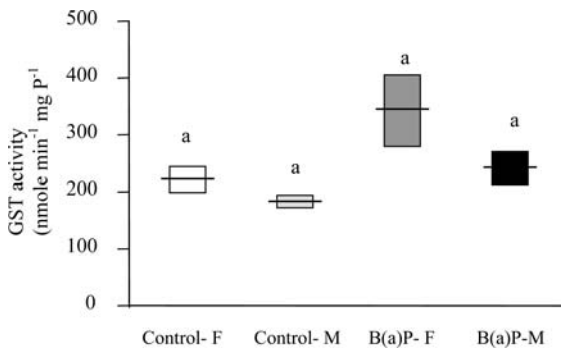

Figure 4. Induction of hepatic GST enzyme activities in sharptooth catfish (*C. gariepinus*) after 4 days of waterborne exposure to B[a]P (30 μg/l). The results are given as mean ± SE ($n = 5$ or 6). ⊟: Unexposed females; ⊟: unexposed males; ■: exposed females; ■: exposed males. Groups not connected by same letters are significantly different (Tukey–Kramer HSD).

Discussion

Findings from this study indicate increased induction of EROD activities in the gills than in the liver following exposure to waterborne B[a]P, suggesting that the absorbed compound was lar-

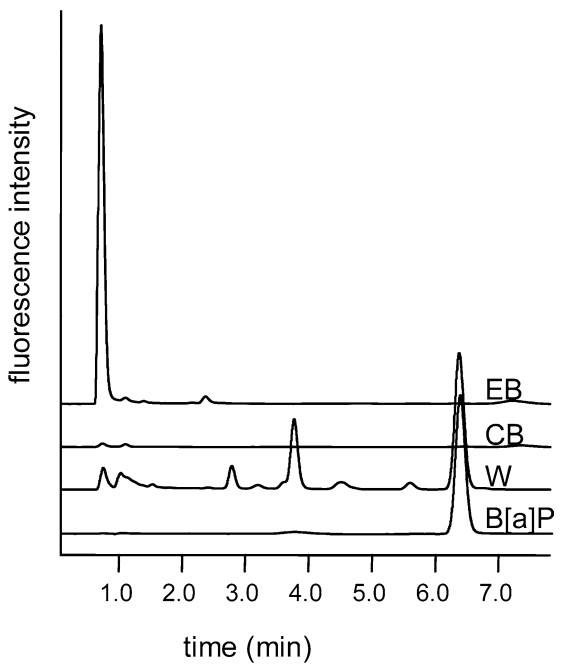

Figure 5. HPLC fluorescence (HPLC/F) chromatogram of a B[a]P standard, a water sample (W) from the exposure tank and bile samples from unexposed (CB) and exposed (EB) fish after 4 days of waterborne exposure to B[a]P. Exitation/emission was monitored at 380/430 nm.

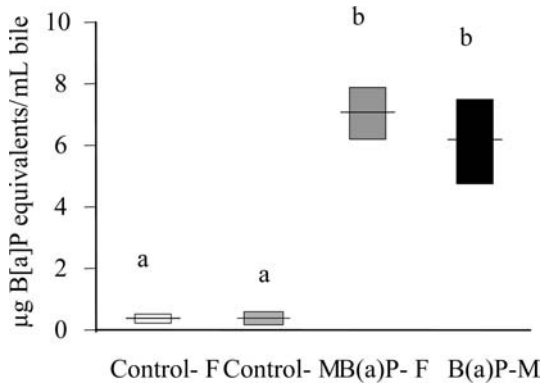

Figure 6. Concentrations of B[a]P-type fluorescent compounds in bile of sharptooth catfish (*C. gariepinus*) after 4 days of waterborne exposure to B[a]P (30 µg/l). The results are given as mean ± SE ($n = 5$ or 6). ⊟: Unexposed females; ⊟: unexposed males; ■: exposed females; ■: exposed males. Groups not connected by same letters are significantly different (Tukey–Kramer HSD).

gely metabolised in the gills and therefore only a small fraction of unmetabolised B[a]P was reaching the liver. This is ascribed to the efficiency of gills in metabolising waterborne organic pollutants and similar findings were reported in previous studies (Levine and Oris, 1999; Jönsson, 2003). According to Carlsson et al. (1999) and Levine and Oris (1999), even a relatively low metabolic capacity in the gills can contribute significantly to prevent accumulation of environmental pollutants in organs such as the liver. The gills thus fulfil a vital role in the overall protection against harmful substances by lowering the total uptake.

The basal gill EROD activities observed in *C. gariepinus* were in the same range as previously reported in several cold-water species (Jönsson et al., 2002). The induced EROD activity in gills of *C. gariepinus* was similar to the response observed in rainbow trout and more than five times higher than levels recorded in Atlantic salmon, Arctic charr, cod and saithe (Jönsson et al., 2003).

The present study shows that the EROD gill filament assay previously developed in rainbow trout (Jönsson et al., 2002) is applicable to tropical fish species such as *C. gariepinus*. The clear EROD induction by B[a]P implies that this fish species can be used to monitor waterborne AhR agonists in the environment.

A relatively higher induction of EROD activities was observed in males compared to the females (Figs. 2 and 3). Since, mature fish were used in this study, the minor sex differences observed in the induction of CYP1A activities could be attributed to higher concentrations of oestrogens in females. Whereas exposure with the oestrogen 17β-oestradiol reduced the total P450 content, some degree of induction of this enzyme was also noticed following exposure to androgens, (Kloepper-Sams and Benton, 1994; Kosmala et al., 1998).

Similar to gill filament EROD activity, levels of FACs showed a significant increase after exposure to waterborne B[a]P. The levels of FACs in bile showed a similar response when measured as B[a]P equivalents/ml bile or as B[a]P equivalents/mg biliverdin. Biliverdin is used to measure relative bile concentrations. In starving fish, bile is retained in the gall bladder whereas both water and small ions are actively transported through the gall bladder epithelium, thus concentrating biliary components (Klaassen and Watkins, 1984). Biliverdin levels observed in *C. gariepinus*, which had been starved for 2 weeks before the experiment, were similar to levels found in English sole after 28 days of starvation in captivity (Collier and Varanasi, 1991).

In this study, GST activities towards CDNB in *C. gariepinus* were not affected following waterborne exposure to B[a]P (Fig. 4). Whereas some investigators detected significant induction in their studies (Andersson et al., 1985; Leaver et al., 1992), others did not (Collier and Varanasi, 1991; Sturm et al., 1999). The GST activity towards CDNB is considered as an integration of overall GST activity. With a few exceptions, all isoforms conjugate CDNB. In mammals, more class-specific substrates exist, but only a few specific substrates for single isoforms have been described (Hayes and Pulford, 1995). As the individual subunits of the dimeric isoforms possess independent activities, and since no truly specific model substrate exist, activity measurements do not provide information on the subunit composition. Thus the results based on this assay are very hard to interpret in fish and this may explain the confusing results obtained by different researchers with respect to PAH-mediated GST induction.

In conclusion, this study shows that CYP1A inducing compounds like B[a]P can be monitored by the gill filament EROD assay under tropical conditions using *C. gariepinus*. Thus, the gill filament EROD assay has proven to be a robust, straightforward and sensitive method for determination of basal and induced EROD activities. Furthermore, this method is a relatively low cost and time saving compared to traditional microsome-based techniques. This makes it a most suitable biomarker in programs designed to monitor PAH type pollution in aquatic systems particularly in countries where advanced laboratory facilities are not always available. It is therefore proposed to utilise CYP1A induction in gills of C. *gariepinus* as well as accumulation of biliary FACs as biomarkers in future studies planned to monitor exposure to PAHs in aquatic ecosystems in eastern and southern Africa.

Acknowledgments

This study was kindly funded by the Norwegian Universities' Committee for Development Research and Education (NUFU) PRO 08/2002. The experiment was approved by the Animal Use and Care and Research Committees of the Faculty of Veterinary Science, University of Pretoria. We express our sincere thanks to the technical assistance we received from Mr. Huyser and Mrs. Mulders, University of Pretoria.

References

Andersson, T., Pesonen, M. and Johansson, C. (1985). Differential induction of cytochrome P−45-dependent monooxygenase, epoxide hydrolase, glutathione transferase and UDP glucuronosyltransferase activities in the liver of rainbow trout by β-naphthoflavone or Clophen A50. *Biochem. Pharmacol.* **34**, 3309–14.

Beyer, J., Sandvik, M., Hylland, K., Fjeld, E., Egaas, E., Aas, E., Skåre, J. and Goksøyr, A. (1996). Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus* L.) and Atlantic cod (*Gadus morhua* L.) exposed by caging to polluted sediments in Sorfjorden, Norway. *Aquat. Toxicol.* **36**, 75–98.

Beyer, J., Sandvik, M., Skaare, J.U., Egaas, E., Hylland, K., Waagbo, R. and Goksoy, A. (1997). Time and dose-dependent biomarker responses in flounder (*Platichthys flesus* L.) exposed to benzo[a]pyrene, 2,3,3́,4,4́,5-hexachlorobiphenyl (PCB−156) and cadmium. *Biomarkers* **2**, 35–44.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–54.

Carlsson, C., Pärt, P. and Brunström, B. (1999). 7-Ethoxyresorufin *O*-deethylase induction in cultured gill epithelial cells from rainbow trout. *Aquat. Toxicol.* **47**, 117–28.

Clements, W.H., Oris, J.T. and Wissing, T.E. (1994). Accumulation and food chain transfer of Fluoranthene and Benzo[a]pyrene in *Chironomus riparius* and *Lepomis macrochirus*. *Arch. Environ. Contam. Toxicol.* **26**, 261–6.

Collier, T.K. and Varanasi, U. (1991). Hepatic activities of xenobiotic metabolising enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental contaminants. *Arch. Environ. Contam. Toxicol.* **20**, 462–73.

Datta, M. and Kaviraj, A. (2003). Ascorbic acid supplementation of diet for reduction of deltamethrin induced stress in freshwater catfish *Clarias gariepinus*. *Chemosphere* **53**, 883–8.

Gadagbui, B.K.M. and Goksøyr, A. (1996). Biomarker studies in tilapia (*Oreochromis niloticus*) caged in, and in mudfish (*Clarias anguillaris*) exposed to sediment from a polluted river in Ghana, West Africa. *Marine Environ. Res.* **42**, 275.

Gallagher, E.P., Gross, T.S. and Sheehy, K.M. (2001). Decreased glutathione *S*-transferase expression and activity and altered sex steroids in Lake Apopka brown bullheads (*Ameriurus nebulosos*). *Aquat. Toxicol.* **55**, 223–37.

Goksøyr, A. and Förlin, L. (1992). The cytochrome P−450 system in fish: aquatic toxicology and environmental monitoring. *Aquat. Toxicol.* **22**, 287–312.

Grossbard, M.L., Aas, E., Beyer, J. and Goksøyr, A. (1987). Fixed wavelength fluorescence (FF) of bile as a monitoring tool for polyaromatic hydrocarbon exposure in fish: an evaluation of compound specificity, inner filter effect and signal interpretation. *Biomarkers* **5**, 9–23.

Habig, W.H., Pabst, M.J. and Jacoby, W.B. (1974). Glutathione *S*-transferases. The first enzymic step in mercapturic acid formation. *J. Biol. Chem.* **249**, 7130–9.

Hayes, J.D. and Pulford, D.J. (1995). The glutathione *S*-Transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* **30**, 445–600.

Jaward, F.M., Barber, J.L., Booij, K. and Jones, K.C. (2004). Spatial distribution of atmospheric PAHs and PCNs along a north-south Atlantic transect. *Environ. Pollut.* **132**, 173–81.

Jönsson, E.M. (2003). A gill filament EROD assay: development and application in environmental monitoring. Ph.D. Thesis, Uppsala University, Sweden.

Jönsson, E.M., Abrahamson, A., Brunstrom, B., Brandt, I., Ingebrigtsen, K. and Jørgensen, E.H. (2003). EROD activity in gill filaments of anadromous and marine fish as a biomarker of dioxin-like pollutants. *Comp. Biochem. Physiol.* **136**, 235–43.

Jönsson, E.M., Brandt, I. and Brunstrom, B. (2002). Gill filament-based EROD assay for monitoring waterborne dioxin-like pollutants in fish. *Environ. Sci. Technol.* **36**, 3340–4.

Klaassen, C. and Watkins, J.B. III (1984). Mechanisms of bile formation, hepatic uptake, and biliary excretion. *Pharmacol. Rev.* **36**, 1–67.

Kloepper-Sams, P.J. and Benton, E. (1994). Exposure of fish to biologically treated bleached-kraft effluent: II. Induction of hepatic cytochrome P4501A in mountain whitefish (*Prosopium williamsoni*) and other species. *Environ. Toxicol. Chem.* **13**, 1483–96.

Kosmala, A., Migeon, B., Flammarion, P. and Garric, J. (1998). Impact assessment of a wastewater treatment plant effluent using the fish biomarker Ethoxyresorufin-*O*-deethylase: field and on-site experiments. *Ecotoxicol. Environ. Saf.* **41**, 19–28.

Krahn, M.M., Brown, D.W., Collier, T.K., Friedman, A.J., Jenkins, R.G. and Malins, D.C. (1980). Rapid analysis of naphthalene and its metabolites in biological systems: determination by high performance liquid chromatography/fluorescence detection and by plasma desorption/chemical ionisation mass spectrometry. *J. Biochem. Biophys. Meth.* **2**, 233–46.

Krahn, M.M., Burrows, D.G., Macleod, W.D. and Malins, D.C. (1987). Determination of individual metabolites of aromatic compounds in hydrolyzed bile of English sole (*Parophrys vetulus*) from polluted sites in Puget sound, Washington. *Arch. Environ. Contam. Toxicol.* **16**, 511–22.

Krahn, M.M., Kittle, L.J. and Macleod, W.D. (1986). Evidence for exposure of fish to oil spilled into the Columbia river. *Marine Environ. Res.* **34**, 237–41.

Leaver, M.J., Pirrit, L. and George, S.G. (1992). Expression and tissue distribution of Plaice glutathione *S*-transferase A. *Marine Environ. Res.* **34**, 237–41.

Levine, S.L. and Oris, J.T. (1999). CYP1A expression in liver and gill of rainbow trout following waterborne exposure: implications for biomarker determination. *Aquat. Toxicol.* **46**, 279–87.

Lien, N.T., Adriaens, D. and Janssen, C.R. (1997). Morphological abnormalities in African catfish (*Clarias gariepinus*) larvae exposed to malathion. *Chemistry* **35**, 1475–86.

Mbongwe, B., Legrand, M., Blais, J.M., Kimpe, L.E., Ridal, J.J. and Lean, D.R. (2003). Dichlorodiphenyltrichloroethane in the aquatic ecosystem of the Okavango Delta, Botswana, South Africa. *Environ. Toxicol. Chem.* **22**, 7–19.

Mccormick, S.D. and Bern, H.A. (1989). *In vitro* stimulation of Na^+–K^+-ATPase activity and ouabain binding by cortisol in coho salmon gill. *Am. J. Physiol.* **256**, R707–15.

Mitchell, A.E., Lakritz, J. and Jones, A.D. (2000). Quantification of individual glutathione *S*-transferase isozymes in hepatic and pulmonary tissues of naphthalene-tolerant mice. *Arch. Toxicol.* **74**, 215–21.

Nguyen, L.T., Janssen, C.R. and Volckaert, F.A. (1999). Susceptibility of embryonic and larval African catfish (*Clarias gariepinus*) to toxicants. *Bull. Environ. Contam. Toxicol.* **62**, 230–7.

Olajire, A.A., Altenburger, R., Küster, E. and Brack, W. (2005). Chemical and ecotoxicological assessment of polycyclic aromatic hydrocarbon – contaminated sediments of the Niger Delta, Southern Nigeria. *Sci. Tot. Environ.* **340**, 123–36.

Payne, J.F., Fancey, L.L., Rahimtula, A.D. and Porter, E.L. (1987). Review and perspective on the use of mixed function oxygenase enzymes in biological monitoring. *Comp. Biochem. Physiol.* **86**, 233–45.

Sturm, A., Hodson, P.V., Carey, J.H. and Hansen, P. (1999). Hepatic UDP-glucuronosyltransferase in rainbow trout (*Oncorhynchus mykiss*) and preliminary assessment of response to pulp mill cooking liquor. *Bull. Environ. Contam. Toxicol.* **62**, 608–15.

Theron, J., Prinsloo, J.F. and Schoonbee, H.J. (1991). Investigations into the effects of concentration and duration of exposure to formalin and malachite green on the survival of the larvae and juveniles of the common carp (*Cyprinus carpio* L). and the sharptooth catfish (*Clarias gariepinus*) (Burchell)Onderstep. *J. Vet. Res.* **58**, 245–51.

Timmers, R.J., Granneman, J.C., Lambert, J.G. and Van Oordt, P.G. (1988). Estrogen−2-hydroxylase in the brain of the male African catfish, *Clarias gariepinus*. *Gen. Comp. Endocrin.* **72**, 190–203.

Viljoen, A., Steyn, G.J., Van Vuren, J.H. and Wade, P.W. (2003). Zinc effects on the embryos and larvae of the sharptooth catfish, *Clarias gariepinus* (Burchell, 1822). *Bull. Environ. Contam. Toxicol.* **70**, 1022–27.