Induction of Hepatic Cytochrome P-450 1A in Pikey Bream (Acanthopagrus berda) Collected from Agricultural and Urban Catchments in Far North Queensland

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A variety of sources of organic contaminants to the Great Barrier Reef lagoon and near-shore environment exist including boating activity, agriculture and urban run-off. Cytochrome P-450 1A activity as measured by ethoxyresorufin O-deethylase (EROD) activity has been widely used as an indicator of the exposure of fish to organic contaminants such as polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH) and some organochlorine pesticides. This study demonstrates the successful application of EROD measurements in a common Australian tropical estuarine fish species, Acanthopagrus berda (Pikey Bream), to identify areas under potential stress from organic contaminants. Fish were captured from four creeks draining agricultural land, a creek draining urban land and two creeks with less disturbed catchments. Significant induction of cytochrome P450-1A was observed in fish captured from Ross Creek (urban catchment, 7.4-fold) and Cromarty Creek (agricultural catchment, 6.4-fold). Increased activity was also observed in fish captured from other creeks draining agricultural land (Plantation Creek, Victoria Creek, Seymour River, 1.9–2.6-fold) as compared to those captured from creeks in undisturbed catchments (Baldy Creek, Fisher Creek, 67–114 pmol/min/mg protein). © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Concern over the environmental impact of continuously increasing anthropogenic land usage (both urban and agricultural) in northern Australia, on the adjacent marine environment has increased in recent years. Numerous studies have focussed on aspects of nutrient and sediment contamination (e.g. Yellowlees, 1991; Furnas et al., 1995) while comparatively few have examined the distribution of organic contaminants such as pesticides (Rayment et al., 1997) and petroleum hydrocarbons (Smith et al., 1985; Sandstrom, 1988). Fewer still have investigated biological aspects of chemical contamination. These have largely focussed on chemical residues in biological tissue (e.g. von Westernhagen and Klumpp, 1995; Russell et al., 1996; Rayment et al., 1997) although Klumpp and von Westernhagen (1995) described abnormal development in fish larvae captured in coastal regions of the Great Barrier Reef Lagoon.

Cytochrome P-450 enzymes are the primary group of oxidative enzymes involved in metabolism of xenobiotic compounds. Exposure to xenobiotic compounds results in induction, or increased synthesis, of particular cytochrome P-450 enzymes. Of the cytochrome P-450 enzymes, the cytochrome P-450 1A sub-family is especially sensitive to induction by a range of organic contaminants, including petroleum hydrocarbons, PCBs, dioxins, furans, organochlorine pesticides and PAHs (e.g. Payne et al., 1987; Goksoyr and Forlind, 1992; Holdway et al., 1995; Denison and Heath-Pagliuso, 1998). As such, induction of cytochrome P-450 1A in fish has been widely used as an indicator of biological exposure to...
organic contaminants in aquatic systems (e.g. Burns, 1976; Stegeman et al., 1986; Galgani et al., 1991; Livingston et al., 1993; Vrolijk et al., 1994; Collier et al., 1998 and many others). Measurement of ethoxyresorufin O-deethylase (EROD) activity is specific for cytochrome P-450 1A. Catalytic assays such as EROD provide a rapid and relatively inexpensive means for assessment of fish exposure to organic contaminants. Limited studies of induction of cytochrome P-450 1A in fish have been conducted in Australia (e.g. Ahokas et al., 1994; Holdway et al., 1994; Brumley et al., 1995) and no studies have previously been conducted in tropical Australia. This study examines the induction of the cytochrome P-450 1A enzymes in a common tropical estuarine fish species, *Acanthopagrus berda*, collected from creeks draining urban land, land with no significant disturbance and agricultural land in two major sugar-cane regions – the Herbert and Burdekin regions.

**Materials and Methods**

**Sample sites**

The Herbert and Burdekin River regions are two significant sugarcane growing regions which together produce approximately 32% of Australia’s sugar. Sugarcane is grown largely on the coastal floodplains of the two rivers, covering approximately 65,000 and 85,000 ha in the Herbert and Burdekin regions, respectively. Fish were collected from two creeks in each of the Herbert River and Burdekin/Haughton River catchments which drain the coastal floodplain and have significant areas under sugarcane; one creek with significant urban land-use (Ross Creek) and two creeks with no significant agricultural or urban activity (Baldy Creek (Cape Ferguson) and Fisher Creek (Hinchinbrook Channel) (Fig. 1). More detailed description of the individual creeks are given below and summarized in Table 1. Approximate percentage land use was determined from maps and Global Information System (GIS) databases.

**Sugarcane catchments**

Sugarcane is the dominant land-use in Plantation Creek (Burdekin) and Victoria Creek (Herbert) catchments and comprises a significant proportion of the area in the Seymour River and Cromarty Creek catchments (Table 1). Most of the caneland drained by Plantation Creek, Victoria Creek and Cromarty Creek is land which has been used for sugarcane farming for >60 years while a greater proportion of the sugarcane land in the Seymour River catchment is relatively recent (<20 years). Recreational boating is popular in all creeks and boat ramps are located in Cromarty Creek, Plantation Creek and Victoria Creek. Additionally, commercial fishing activities (Barramundi) occur in Victoria Creek.

![Fig. 1 Map showing the location of sample sites and major river catchment boundaries.](image)
Livers were stored at −80°C until analysis. Microsomes were prepared by homogenizing the liver in 0.1 M phosphate buffer (pH 7.4) followed by sequential centrifugation. All steps were performed at 4°C. The final centrifugation was for 60 min at 100,000 g. Microsomes were resuspended in phosphate buffer containing 20% glycerol and stored at −80°C until analyses.

Cytochrome p-450 content
Cytochrome P-450 content was determined by the dithionite reduced difference spectral method of Matsubara et al. (1976) with modifications by Rutten et al. (1987). Briefly, microsomal suspensions were diluted 1/10 in 0.1 M phosphate buffer containing 20% glycerol at room temperature, and bubbled with carbon monoxide for 30 s. After an additional 2 min for stabilization, the baseline spectrum was recorded. Sodium dithionite (final concentration, 4.58 mM) was added to the sample cuvette and after 3 min, the spectrum between 400 and 500 nm was recorded using a Shimadzu UV 3000 dual wavelength/double beam recording spectrophotometer. An extinction coefficient of 104 M−1 cm−1 was used to calculate total P-450 content (Matsubara et al., 1976).

Ethoxyresorufin O-dethylase (EROD) analysis
EROD activity was determined using a fluorescence method modified from Burke and Mayer (1975). All assays were conducted at 35°C and pH 7.6. The incubation mixture consisted of an NADPH regenerating system (10 mM magnesium chloride, 200 mM potassium chloride, 6 mM glucose-6-phosphate, 1.25 mM NADP and 100 units G-6-dehydrogenase, 250 µl), 0.525 ml Tris buffer (pH 7.6), 100 µl albumin and 100 µl of ethoxyresorufin substrate. Reaction was started with the addition of 25 µl of the microsomal preparation, incubated for 5 min prior to stopping the reaction with 2.5 ml of methanol. The resulting precipitate was centrifuged (2000 g, 5 min) prior to determination of resorufin on a Hitachi F-4010 fluorescence spectrophotometer. Fluorescence was determined at EX/EM wavelength of 530/584 nm. Assays were performed in triplicate. Resorufin concentration was calculated from a standard regression after correction of readings for blank fluorescence.

All enzyme activities were expressed as a rate per mg of protein or nmol P-450. Normalization of EROD activity to total P-450 content gives an indication of the relative contribution of cytochrome P-450 1A to total P-450 content, i.e., a higher value can suggest a relative
enrichment of cytochrome P-450 1A (Stegeman et al., 1997). Protein assays were performed on the microsomal suspension using the method of Lowry et al. (1951).

Statistical analyses
The log transformed activities (EROD normalized to protein and total cytochrome P-450, and total cytochrome P-450 normalized to protein) were analysed using a general linear model which included the effects: disturbance regime (agriculture, urban and undisturbed), sexual reproductive status (active male, active female, inactive male, inactive female), lesions (present, absent) and catchment (Seymour River, Victoria Creek, Plantation Creek, Cromarty Creek, Ross Creek). The differences in activities, due to the main effects of interest (disturbance regime and catchment), were estimated using contrasts. The data were analysed on the transformed scale as the assumptions of normality and homogeneity of variance were not valid on the untransformed scale.

Effects were considered at the 5% level of significance and all results presented on the untransformed scale.

Results and Discussion

Table 2 provides descriptive statistics on the fish captured. Significant effects of sexual reproductive status on EROD activity were observed (Table 3). Females, sexually active fish (both males and females) and fish with external lesions showed a lower and more variable activity (unpub. data). No significant effects were observed on total cytochrome P-450 content. Values presented in this paper for comparative EROD activity are those for sexually inactive and not obviously sick, male fish (Tables 4 and 5). In addition to biological variables (such as sex, age, spawning status), habitat variables, primarily temperature, can influence activity of the cytochrome P-450 1A in fish (Stegeman and Chevion, 1980; Koivussaari et al., 1981; Jiminez and Burtis, 1989). Water quality parameters were not collected during this study.

However, the variability of these parameters in the different creek systems over the short time fish were collected (1 week) is unlikely to significantly effect cytochrome P450 1A response.

Effect of catchment disturbance on cytochrome P-450 1A activity

Fish captured from catchments disturbed by agricultural or urban activity showed significantly elevated EROD activity as compared to fish captured from undisturbed catchments (Table 4). Additionally, fish captured from Ross Creek (urban catchment) showed a significantly higher activity (2.6-fold) as compared to those captured from creeks draining sugarcane land. A relative increase of EROD activity normalized to total cytochrome-P-450 as compared to EROD activity normalized to total protein occurred in fish captured from sugarcane catchments (3-fold and 2.5-fold increase, respectively, relative to undisturbed catchments, Table 4). This is perhaps not surprising given the wide range of organic contaminants present in the sediments of this system (Smith et al., 1985; Kross, 1997; Inglis and Kross, 2000) and therefore, potential induction of other cytochrome P-450 enzymes (Van der Oost et al., 1994; Stegeman et al., 1997). Interestingly, fish captured from sugarcane catchments showed a significantly lowered total cytochrome P-450 content as compared to fish captured from undisturbed or urban catchments. This is unusual as increased EROD activity is typically coincident with increased total cytochrome P-450 content as a result of increased production of cytochrome P-450 enzymes. Fish captured from the urban catchment showed slightly elevated, although not statistically significant different, amount of total cytochrome P-450 as compared to fish captured from the undisturbed catchments.

Cytochrome P-450 activity in individual catchments

Undisturbed catchments. Despite the absence of significant disturbance in the Fisher Creek catchment, its location within Hinchinbrook Channel and sediment dynamics within the channel suggest that fish captured in Fisher Creek are likely to be exposed to any sediment and associated (Wolanski et al., 1990) contaminants originating from the Herbert River and surrounding...
creeks. Additionally, higher recreational boating activity occurs in Fisher Creek as compared to Baldy Creek. Thus, it is not surprising to observe that EROD activity in Fisher Creek is elevated as compared to Baldy Creek. However, it was surprising that this increase in activity was significant (Table 5). In subsequent discussion, all increases of EROD activity normalized to total protein or total cytochrome P-450 are expressed relative to the EROD activity in fish captured from Baldy Creek.

Sugarcane catchments. EROD activity in fish captured from all creeks draining sugarcane land showed a significant increase in activity as compared to fish captured from Baldy Creek. A 2.1–2.9-fold increase in activity normalized to total protein or 2.5–4.6-fold increase in EROD activity normalized to total cytochrome P-450 was observed for fish captured from Plantation Creek, Victoria Creek and Seymour River. EROD activity in fish captured from Seymour River

### TABLE 3

Overall significance of effects (p-values) of disturbance regime, catchment (disturbed catchments only), sexual reproductive status, and lesions, on EROD activity (normalized to protein and total cytochrome P-450) and total cytochrome P-450 normalized to protein.

<table>
<thead>
<tr>
<th>Effect</th>
<th>EROD activity (pmol/min/mg protein)</th>
<th>EROD activity (nmol/min/nmol P450)</th>
<th>Total P-450 (nmol P450/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disturbance regime</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0005</td>
</tr>
<tr>
<td>Catchment</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0245</td>
</tr>
<tr>
<td>Sex and reproductive status</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.6537</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.0266</td>
<td>0.4062</td>
<td>0.0844</td>
</tr>
</tbody>
</table>

### TABLE 4

Estimates of EROD activity (normalized to total protein and total P-450) and P-450 in sexually inactive male fish collected from undisturbed, sugarcane and urban catchments and the ratio of disturbed catchments relative to the undisturbed catchments for each of these activities.

<table>
<thead>
<tr>
<th>Catchment grouping</th>
<th>No. of fish</th>
<th>EROD activity (pmol/min/mg protein) mean (95% CI)</th>
<th>Ratio (95% CI)</th>
<th>EROD activity (nmol/min/nmol P450) mean (95% CI)</th>
<th>Ratio (95% CI)</th>
<th>Total P-450 (nmol P450/mg protein) mean (95% CI)</th>
<th>Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undisturbed</td>
<td>24</td>
<td>88 (71–107)</td>
<td></td>
<td>0.41 (0.33–0.52)</td>
<td></td>
<td>0.21 (0.18–0.25)</td>
<td></td>
</tr>
<tr>
<td>Sugarcane</td>
<td>57</td>
<td>217 (188–249)</td>
<td>2.6 (1.9–3.5)</td>
<td>1.3 (1.1–1.5)</td>
<td>3.1</td>
<td>0.17 (0.15–0.19)</td>
<td>0.81</td>
</tr>
<tr>
<td>Urban</td>
<td>15</td>
<td>564 (429–742)</td>
<td>6.4 (4.6–9.1)</td>
<td>2.1 (1.6–2.9)</td>
<td>5.2</td>
<td>0.26 (0.22–0.32)</td>
<td>1.2</td>
</tr>
</tbody>
</table>

### TABLE 5

Estimates of EROD activity (normalized to total protein and total P-450) and P-450 content in sexually inactive male fish collected from individual catchments and the ratio of each catchment relative to Baldy Creek for each activity.

<table>
<thead>
<tr>
<th>Catchment</th>
<th>No. of fish</th>
<th>EROD activity (pmol/min/mg protein)</th>
<th>Ratio (95% CI)</th>
<th>EROD activity (nmol/min/nmol P450)</th>
<th>Ratio (95% CI)</th>
<th>Total P-450 (nmol P450/mg protein)</th>
<th>Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baldy</td>
<td>14</td>
<td>67 (51–87)</td>
<td></td>
<td>0.31 (0.22–0.40)</td>
<td></td>
<td>0.22 (0.18–0.27)</td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>7</td>
<td>114 (84–155)</td>
<td>1.7 (0.9–2.5)</td>
<td>0.57 (0.41–0.80)</td>
<td>1.9</td>
<td>0.20 (0.16–0.25)</td>
<td>0.9</td>
</tr>
<tr>
<td>Victoria</td>
<td>13</td>
<td>196 (150–258)</td>
<td>(2.0–4.3)</td>
<td>1.4 (1.0–1.9)</td>
<td>4.6</td>
<td>0.14 (0.11–0.17)</td>
<td>0.6</td>
</tr>
<tr>
<td>Seymour</td>
<td>10</td>
<td>161 (118–220)</td>
<td>(1.6–3.6)</td>
<td>0.75 (0.53–1.0)</td>
<td>2.5</td>
<td>0.22 (0.17–0.27)</td>
<td>1.0</td>
</tr>
<tr>
<td>Plantation</td>
<td>17</td>
<td>142 (111–181)</td>
<td>(1.5–3.0)</td>
<td>0.94 (0.72–1.2)</td>
<td>3.2</td>
<td>0.15 (0.12–0.18)</td>
<td>0.7</td>
</tr>
<tr>
<td>Cromarty</td>
<td>17</td>
<td>492 (380–637)</td>
<td>(5.1–10.6)</td>
<td>2.6 (2.0–3.4)</td>
<td>8.6</td>
<td>0.19 (0.16–0.23)</td>
<td>0.8</td>
</tr>
<tr>
<td>Ross</td>
<td>15</td>
<td>564 (429–742)</td>
<td>(5.8–12.3)</td>
<td>2.1 (1.6–2.9)</td>
<td>7.1</td>
<td>0.21 (0.22–0.32)</td>
<td>1.2</td>
</tr>
</tbody>
</table>

A Values within a column without a common superscript letter are significantly different (p < 0.05).
and Plantation Creek was not significantly different from those captured from Fisher Creek. A general trend of decreased total cytochrome P-450 content was observed in all sugarcane creeks although it was only significant for Plantation Creek and Victoria Creek (both of which have a longer history of agricultural land usage). Remarkably high EROD activity was observed in fish captured from Cromarty Creek (7.3–8.6-fold increase, Table 5) and was similar to EROD activity observed in fish captured from the urban catchment. This result was unexpected as the Cromarty Creek catchment has a relatively smaller area disturbed by sugarcane growing than most of the other sugarcane catchments. Additionally, boating activity in Cromarty Creek is no greater than that in creeks draining the other sugarcane catchments. Cromarty Creek however has an unusual hydrological regime. It is located in an extensive area of estuarine wetland and restriction of tidal waters entering and leaving the wetland area combined with a limited freshwater input has resulted in the formation of a slowly flushed local sedimentary depositional basin (Blackman, pers. comm). This may influence the distribution of contaminants in the system.

Urban catchment. Fish captured from Ross Creek showed significantly elevated EROD activity as compared to fish captured from Baldy Creek (7.1–8.4-fold increase, Table 5) and a slight, though not significantly increased total cytochrome P-450. EROD induction in fish captured from Ross Creek was not unexpected given known contamination of the sediment (Kross, 1997; Inglis and Kross, 2000).

Sources of inducers of cytochrome P-450 1A in Queensland catchments.

The observed induction of cytochrome P-450 1A in A. herda captured in catchments disturbed by agricultural or urban activity, suggest they have been exposed to inducing agents as a result of disturbance. A variety of sources of inducers to the study catchments exist including boating activity, agriculture and urban run-off. The first step in elucidating the sources of inducers of cytochrome P-450 1A is the identification of the inducers. A wide range of chemicals can induce cytochrome P-450 1A. The best known inducers are planar PCBs, chlorinated dibenzodioxins and furans and PAHs, primarily those with 3 or more benzene rings (Stegeman and Hahn, 1994). A recent study also identified a wide range of ‘non-classical’ inducers including brevetoxin and the pesticide carbaryl (Denison and Heath-Pagliuso, 1998). Given this wide range of chemical inducers, it can be difficult to determine if particular classes of compounds are primarily responsible for observed induction at a given location (Collier et al., 1998). Sediments are generally considered to be the major source of cytochrome P-450 1A inducers as these chemicals are typically bound to sediment particles.

Sediment sampling conducted in the study creeks found low concentrations of PAHs and organochlorine insecticide residues detected in sediment collected in all creeks except Ross Creek in which high concentrations of PAHs and organochlorine insecticides were found (unpublished data). A recent study suggested that PAH exposure was likely to be the major factor in environmental induction of cytochrome P-450 1A in fish collected from coastal regions of the USA (Gardinali and Wade, 1998). The presence of high concentrations of PAHs in Ross Creek sediments suggests PAHs are the cause of induction in fish collected from this location. With the exception of fish captured from Cromarty Creek, induction in agricultural catchments may also be due to low concentrations of PAHs. The remarkably high EROD activity observed in fish captured from Cromarty Creek was unexpected and may suggest the importance of local hydrological regimes in determining exposure of fish to contaminants and warrants further investigation. Whether induction of cytochrome P-450 1A is a result of transient or chronic exposure to inducing agents can also aid in the identification of the inducers. Removal of fish from the source of inducers can result in return of cytochrome P-450 1A to basal levels over a period of 3 weeks (Woodin et al., 1997) although this time-frame will depend on the nature of the inducing agent. Collection of fish from the study sites at other time points would provide an indication on the nature of exposure to and the identity of the inducers.

Off-site movement of soil and associated contaminants is generally considered to be the major source of contaminants to the north Queensland coastal environment (e.g. Bramley and Johnson, 1996; Furnas and Brodie 1996; Hunter et al., 1996; Mitchell et al., 1996), although none of these studies have examined the distribution of PAHs. The major source of PAHs to the coastal environment surrounding heavily urbanized areas in the United States was believed to atmospheric deposition of PAHs formed from the combustion of fossil fuels (Collier et al., 1998). While the coastal regions of North Queensland cannot be considered to be heavily urbanized, burning of sugarcane prior to harvest in some regions and subsequent atmospheric deposition of PAHs and PCDD/PCDFs could constitute a significant source of cytochrome P-450 1A inducers to the adjacent coastal environment (Mueller et al., 1996a,b). In this study, fish were collected outside the sugarcane harvesting season and the contribution of contaminants derived from this source to the observed cytochrome P-450 activity is likely to be small. There may however, have been some contribution by atmospheric deposition from volatilized residues derived from urban and other agricultural land use (e.g. pesticides, fuel oil, diesel and gasoline combustion. A previous study of PAH in sediments of the GBR suggested that boating activity was the major source of PAHs to the sediment (Smith et al., 1985). Recreational boating is a popular activity in
many of the study creeks and commercial fishing activities also occur in Victoria Creek and as such, may represent an important source of inducers in the study creeks.

**Implications for the monitoring of the Great Barrier Reef Lagoon coastal environment**

Induction of cytochrome P-450 1A in fish has been widely used as an indicator of biological exposure to organic contaminants in aquatic systems and has been incorporated into at least one monitoring programme (Collier et al., 1998). However, species specific differences in cytochrome P-450 1A activity and sensitivity to inducing agents occur. This confounds comparison of the relative induction observed in *A. berda* to that observed in other studies using different species and under different degrees of pollution. Up to 100-fold differences in levels of EROD activity between different species captured from one location can be observed (e.g. Van der Oost et al., 1991; Spies et al., 1996; Stegeman et al., 1997). Within one species the relative increase in EROD can range between 2- and 30-fold (e.g. Stegeman et al., 1996; Vroljik et al., 1993; Ahokas et al., 1994; Holdway et al., 1995; Spies et al., 1996; Flammaroni and Garric, 1998) for sites considered to be low to highly polluted. Thus, in order to use the relative induction of cytochrome P-450 1A in *A. berda* as an indicator of the degree of pollution, further research into the nature of response in this species as it is influenced by biological and habitat variables is necessary. Despite this, cytochrome P-450 1A response in fish provides a rapid and relatively cheap ‘first-cut’ assessment of the exposure fish to a range of organic contaminants. Over time and as further information is obtained, temporal trends may be observed and related to the condition of the environment. This study provides preliminary baseline information on the cytochrome P-450 1A response in *A. berda* as a biomonitoring tool for the coastal environment of Queensland and Northern Australia.

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