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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).

Keywords: cytochrome P-450; EROD; PAH; organochlorine; fish; tropical Australia; agriculture.

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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 Förlin, 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*-dethylase (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 sugarcane 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.

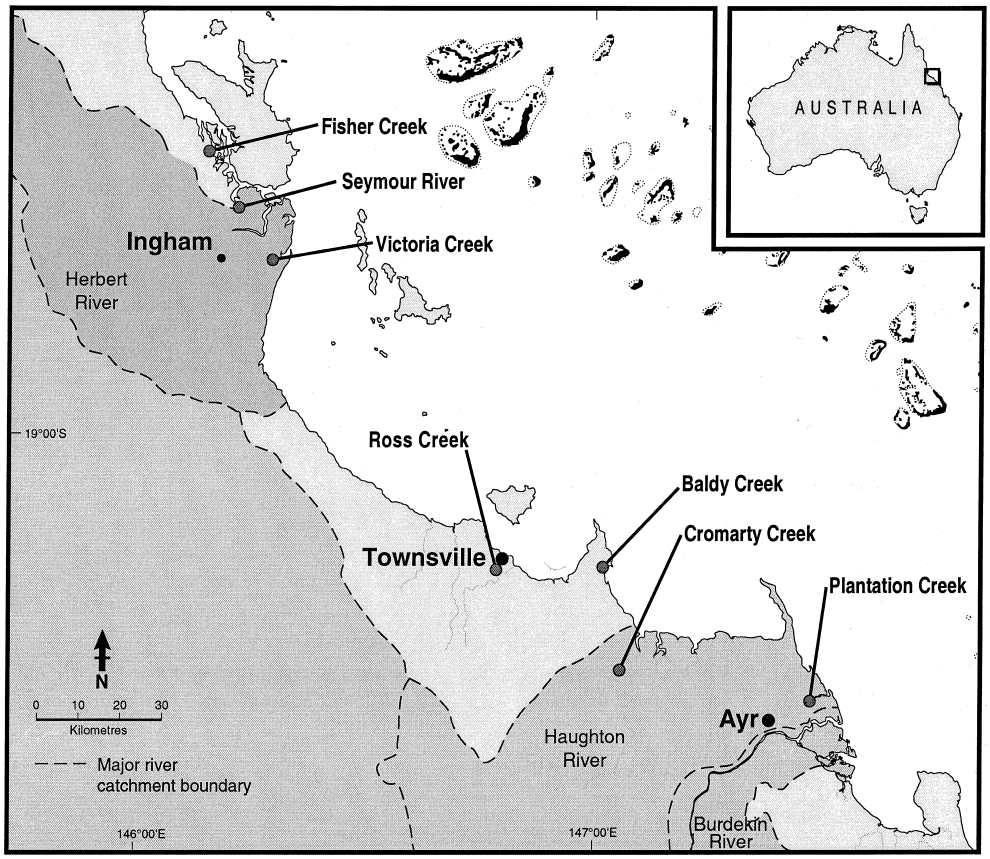

Fig. 1 Map showing the location of sample sites and major river catchment boundaries.

TABLE 1

Description of land use and general characteristics of study catchments.

Catchment	Sub-catchment	Sub-catchment grouping	Approx area (km^2)	Predominant land-use (% of catchment area)	Vegetation[a] (%)	Wetland (%)	Mangrove (%)	Other activities occurring within creek system
Baldy Creek		Undisturbed	40		80		20	
Fisher Creek		Undisturbed	40		70		30	Recreational boating
Herbert River	Victoria	Sugarcane	110	Sugarcane (70)	2	7	20	Commercial fishing, Recreational boating
Herbert River	Seymour	Sugarcane	80	Sugarcane (35)	38	2	20	Recreational boating
Haughton River	Cromarty	Sugarcane	45	Sugarcane (40)	2	60		Recreational boating
Burdekin River	Plantation	Sugarcane	155	Sugarcane (70) Urban (6)		16	12	Recreational boating
Ross River	Ross Creek	Urban	25	Urban (90)	10			Shipping port, Recreational boating

[a] Vegetation – Eucalypt/Melaleuca forest.

Undisturbed catchments

Baldy Creek is a small tidal creek ~2 km in length, located in Bowling Green Bay National Park. No significant recreational boating activities occur in this creek. The Fisher Creek catchment is adjacent to the Herbert River catchment and includes the coastal hills of the Cardwell Range. Fisher Creek drains into the extensive interconnected mangrove swamp of Hinchinbrook Channel. Fisher Creek and Hinchinbrook Channel are popular recreational boating areas and a popular boat ramp is located in Fisher Creek.

Urban catchment

Ross Creek drains through the city of Townsville. The catchment area is predominantly urban land although a limited area of mangrove swamp exists. A man-made lake system that receives stormwater from the surrounding urban area discharges into the top of the creek. Significant commercial and recreational boating activity occurs within Ross Creek due to the presence of a marina, operation of a ferry service and the presence of a large commercial Port facility at the creek mouth.

Fish collection

Fish were captured by hook and line. Immediately after capture, fish were killed, livers dissected out and frozen in liquid N_2. Fish were sexed and their lengths were measured. Gutted weight was determined in the laboratory. Fish were captured over the period 20–29 April 1999. Rainfall prior to the collection period suggested that there would be a moderate freshwater input to the creeks.

Microsome preparation

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 mM^{-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) suggested an enrichment of cytochrome P-450 1A. In contrast, fish captured from the urban catchment showed a decrease in the relative response of EROD activity normalized to total cytochrome P-450 (5.2-fold compared to 6.4-fold increase 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

TABLE 2

Summary statistics of *A. berda* captured from study catchments.

Catchment	Fish captured total (females)	Gutted weight (g) mean ± SD	Length (mm) mean ± SD	Additional information
Baldy	18 (4)	132 ± 27	174 ± 12	
Fisher	22 (5)	111 ± 27	168 ± 13	Some fish collected from a spawning aggregation
Victoria	16 (1)	113 ± 32	170 ± 13	Two fishes with external lesions
Seymour	14 (0)	139 ± 36	174 ± 14	Four fishes with external lesions
Cromarty	17 (0)	105 ± 25	166 ± 12	
Plantation	20 (3)	105 ± 22	162 ± 13	
Ross	15 (0)	102 ± 16	163 ± 9	

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.

Effect	EROD activity (pmol/min/mg protein)	EROD activity (nmol/min/nmol P450)	Total P-450 (nmol P450/protein)
Disturbance regime	0.0001	0.0001	0.0005
Catchment	0.0001	0.0001	0.0245
Sex and reproductive status	0.0001	0.0001	0.6537
Lesions	0.0266	0.4062	0.0844

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.[A]

Catchment grouping	No. of fish	EROD activity (pmol/min/mg protein) mean (95% CI) (min, max)	Ratio (95%CI)	EROD activity (nmol/min/nmol P450) mean (95% CI) (min, max)	Ratio (95% CI)	Total P-450 (nmol P450/protein) mean (95% CI) (min, max)	Ratio (95% CI)
Undisturbed	24	88 (71–107)[a] (23.2, 229)		0.41 (0.33–0.52)[a] (0.11–0.52)		0.21 (0.18–0.25)[a] (0.12, 0.40)	
Sugarcane	57	217 (188–249)[b] (45, 760)	2.6 (1.9–3.5)	1.3 (1.1–1.5)[b] (0.32, 5.36)	3.1 (2.3–4.0)	0.17 (0.15–0.19)[b] (0.03, 0.37)	0.81 (0.67–1.0)
Urban	15	564 (429–742)[c] (193, 1074)	6.4 (4.6–9.1)	2.1 (1.6–2.9)[c] (0.91, 4.3)	5.2 (3.6–7.5)	0.26 (0.22–0.32)[a] (0.14, 0.40)	1.2 (1.0–1.6)

[A] Values within a column without a common superscript letter are significantly different ($p < 0.05$).

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.[A]

Catchment	No. of fish	EROD activity (pmol/min/mg protein) mean (95% CI) (min, max)	Ratio (95%CI)	EROD activity (nmol/min/nmol P450) mean (95% CI) (min, max)	Ratio (95% CI)	Total P-450 (nmol P450/mg protein) mean (95% CI) (min, max)	Ratio (95% CI)
Baldy	14	67 (51–87)[a] (23, 129)		0.31 (0.22–0.40[a] (0.11, 0.64)		0.22 (0.18–0.27)[a,b] (0.12, 0.40)	
Fisher	7	114 (84–155)[b] (55, 229)	1.7 (0.9–2.5)	0.57 (0.41–0.80)[b] (0.24, 0.92)	1.9 (1.2–8.4)	0.20 (0.16–0.25)[a,b] (0.15, 0.36)	0.9 (0.7–1.2)
Victoria	13	196 (150–258)[c] (107, 379)	2.9 (2.0–4.3)	1.4 (1.0–1.9)[c] (0.59, 2.30)	4.6 (3.1–6.9)	0.14 (0.11–0.17)[c] (0.05, 0.27)	0.6 (0.5–0.8)
Seymour	10	161 (118–220)[b,c] (64, 259)	2.4 (1.6–3.6)	0.75 (0.53–1.0)[b,d] (0.36, 1.46)	2.5 (1.6–3.9)	0.22 (0.17–0.27)[a,b] (0.14, 0.34)	1.0 (0.7–1.3)
Plantation	17	142 (111–181)[b,c] (45, 329)	2.1 (1.5–3.0)	0.94 (0.72–1.2)[c,d] (0.35, 2.06)	3.2 (2.2–4.6)	0.15 (0.12–0.18)[c,d] (0.03, 0.38)	0.7 (0.5–0.9)
Cromarty	17	492 (380–637)[d] (294, 760)	7.3 (5.1–10.6)	2.6 (2.0–3.4)[e] (1.43, 5.36)	8.6 (5.8–13.0)	0.19 (0.16–0.23)[b,d] (0.10, 0.31)	0.8 (0.6–1.1)
Ross	15	564 (429–742)[d] (193, 1074)	8.4 (5.8–12.3)	2.1 (1.6–2.9)[e] (0.901, 4.3)	7.1 (4.7–10.6)	0.21 (0.22–0.32)[a] (0.14, 0.4)	1.2 (0.9–1.6)

[A] Values within a column without a common superscript letter are significantly different ($p < 0.05$).

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

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 are 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. berda* 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*., 1990; Vroljik *et al*., 1993; Ahokas *et al*., 1994; Holdway *et al*., 1995; Spies *et al*., 1996; Flammarion 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* response and demonstrates the potential application of cytochrome P-450 1A induction in *A. berda* as a biomonitoring tool for the coastal environment of Queensland and Northern Australia.

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Ahokas, J. T., Holdway, D. A., Brennan, S. E., Goudey, R. W. and Bibrowska, H. B. (1994) MFO activity in carp (*Cyprinus carpio*) exposed to treated pulp and paper mill effluent in Lake Coleman, Vic., Australia, in relation to AOX, EOX, and muscle PCDD/PCDF. *Environmental Toxicology and Chemistry* **13**, 41–50.

Bramley, R. G. V. and Johnson, A. K. L. (1996) Land use impacts on nutrient loading in the Herbert River. In *Downstream Effects of Land Use*, eds. H. M. Hunter, A. G. Eyles and G. E Rayment, pp. 93–96. Department of Natural Resources, Brisbane, Australia.

Brumley, C. M., Haritos, V. S., Ahokas, J. T. and Holdway, D. A. (1995) Validation of biomarkers of marine pollution exposure in sand flathead using Aroclor 1254. *Aquatic Toxicology* **31**, 249–262.

Burke, M. D. and Mayer, R. T. (1975) Inherent specificities of purified cytochromes P-450 and P-448 toward biphenyl hydroxylation and ethoxyresorufin deethylation. *Drug Metabolism and Disposal* **3**, 245–253.

Burns, K. A. (1976) Microsomal mixed function oxidases in an estuarine fish, *Fundulus heteroclitus* and their induction as a result of environmental contamination. *Comparative Biochemistry and Physiology* **53B**, 443–446.

Collier, T. K., Anulacion, B. F. and Bill, B. D. (1998) Hepatic CYP1A in winter flounder (*Pleuronectes americanus*) along the Northeast Coast: results from the National Benthic Surveillance Project. *Marine Pollution Bulletin* **37** (1–2), 86–91.

Denison, M. S. and Heath-Pagliuso, S. (1998) The Ah receptor: A regulator of the biochemical and toxicological actions of structurally diverse chemicals. *Bulletin of Environmental Contamination and Toxicology* **61**, 557–568.

Flammarion, P. and Garric, J. (1997) Cyprinids EROD activity in low contaminated rivers: a relevant statistical approach to estimate reference levels for EROD biomarker? *Chemosphere* **35** (10), 2375–2388.

Furnas, M. J. and Brodie, J. (1996) Current status of nutrient levels and other water quality parameters in the Great Barrier Reef. In *Downstream Effects of Land Use*, eds. H. M. Hunter, A. G. Eyles and G. E. Rayment, pp. 9–22. Department of Natural Resources, Brisbane, Australia.

Furnas, M., Mitchell, A. W. and Skuza, M. (1995) *Nitrogen and Phosphorous Budgets for the Central Great Barrier Reef Shelf*. A report to the Great Barrier Reef Marine Park Authority, Research Publication No. 36, p. 194.

Galgani., F., Bocquene, G., Lucon, M., Grzebyk, D., Letrouit, F. and Claisse, D. (1991) EROD measurements in fish from the northwest part of France. *Marine Pollution Bulletin* **22** (10), 494–500.

Gardinali, P. R. and Wade, T. L. (1998) Contribution of PAHs, PCBs and PCDD/PCDFs to the total induction equivalents ($\sum$IEs) in mollusks. *Marine Pollution Bulletin* **37** (1–2), 27–31.

Goksoyr, A. and Forlin, L. (1992) The cytochrome P-450 system in fish, aquatic toxicology and environmental monitoring. *Aquatic Toxicology* **22**, 287–312.

Holdway, D. A., Brennan, S. E. and Ahokas, J. T. (1994) Use of hepatic MFO and blood enzyme biomarkers in sand flathead (*Platycephalus bassensis*) as indicators of pollution in Port Phillip Bay, Australia. *Marine Pollution Bulletin* **28** (11), 683–695.

Holdway, D. A., Brennan, S. E. and Ahokas, J. T. (1995) Short review of selected fish biomarkers of xenobiotic exposure with an example using fish hepatic mixed-function oxidase. *Australian Journal of Ecology* **20**, 34–44.

Hunter, H. M., Walton, R. S. and Russell, D. J. (1996) Contemporary water quality in the Johnstone River Catchment. In *Downstream Effects of Land Use*, eds. H. M. Hunter, A. G. Eyles and G. E. Rayment, pp. 339–346. Department of Natural Resources, Brisbane, Australia.

Inglis, G. J. and Kross, J. E. (2000) Evidence for systematic changes in the benthic fauna of tropical estuaries as a result of urbanization. *Marine Pollution Bulletin* **41**, 367–376.

Jimenez, B. D. and Burtis, L. S. (1989) Influence of environmental variables on the hepatic mixed-function oxidase system in bluegill sunfish, *Lepomis machrochirus*. *Comparative Biochemistry and Physiology* **93C**, 11–21.

Klumpp, D. W. and Von Westernhagen, H. (1995) Biological effect of pollutants in Australian tropical coastal waters: embryonic malformations and chromosomal aberrations in developing fish eggs. *Marine Pollution Bulletin* **30**, 158–165.

Koivusaari, U., Harri, M. and Hanninen, O. (1981) Seasonal variation of hepatic bio-transformation in female and male rainbow trout (*Salmo gairdneri*). *Comparative Biochemistry and Physiology* **70C**, 149–157.

Kross, J. (1997) The identification of bioindicators of heavy metal and petroleum hydrocarbon pollution in Ross River. Unpublished honours thesis. James Cook University, Townsville, Qld, Australia.

Livingston, D. R. Lemaire, P., Matthews, A., Peters, L, Bucke, D. and Law, R. J. (1993) Pro-oxidant, antioxidant and 7-ethoxyresorufin *O*-deethylase (EROD) activity responses in liver of dab (*Limanda limanda*) exposed to sediment contaminated with hydrocarbons and other chemicals. *Marine Pollution Bulletin* **26** (11), 602–606.

Lowry, O. H., Rosebrough, A. L., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin Phenol reagent. *Journal of Biological Chemistry* **193**, 143–164.

Matsubara, T., Koike, M., Touchi, A., Tochino, Y. and Sugeno, K. (1976) Quantitative determination of cytochrome P-450 in rat liver homgenate. *Analytical Biochemistry* **75**, 596–603.

Mitchell, A. W, Rheghanzani, J. R., Hunter, H. M. and Bramley, R. G. V. (1996) Water quality and nutrient fluxes from river systems draining to the Great Barrier Reef Marine Park. In *Downstream Effects of Land Use*, eds. H. M. Hunter, A. G. Eyles and G. E. Rayment, pp. 23–34. Department of Natural Resources, Brisbane, Australia.

Mueller, J. F., Sutton, M., Wermuth, U. D., Will, S., Hawker, D. W. and Connell, D. W. (1996a) Burning sugar-cane a potential source for PAHs and PCDD/Fs. In Conference Proceedings Intersect 96. Brisbane, Australia.

Mueller, J. F., Sutton, M., Wermuth, U. D., Will, S., Hawker, D. W. and Connell, D. W. (1996b) Polychlorinated dibenzodioxins and polychlorinated dibenzo-furans in topsoils from northern Queensland with a history of different trash management practices. In *Sugarcane; Research Towards Efficient and Sustainable Management Production*, eds. J. R. Wilson, D. M., Hogarth, J. A. Cambell, and A. J. Garside, pp. 273–274. CSIRO Division of Tropical Crops and Pastures.

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. *Comparative Biochemistry and Physiology* **86C** (2), 233–245.

Rayment, G. E., Moss, A., Mortimer, M. and Haynes, D. (1997) Monitoring of pesticides in Queenslands Aquatic Environment and in the Great Barrier Reef Marine Park. Attachment 1 to Downstream effects of Agricultural Practices (DEAP) 97-1 Information Paper 4.2.

Russell, D. J., Hales, P. W. and Moss, A. (1996) Pesticide residues in aquatic biota from north-east Queensland Coastal streams. *Proceedings of the Royal Society of Queensland,* **106** (1), 23–30.

Rutten, A. A. J. J. L., Falke, H. E., Catsburg, R., Topp, B. J., Blauuboer, I., van Holsteijn, I., Doorn, L. and van Leeuwen. (1987) Inter-laboratory comparison of total cytochrome P-450 and protein determination in rat liver microsomes. *Archives of Toxicology* **61**, 27–33.

Sandstrom, M. W. (1988) Aliphatic hydrocarbons in surface sediments from North Queensland coast and Great Barrier Reef: effects of tropical cyclone Winifred. *Organic Geochemistry* **12** (5), 445–454.

Smith, J. D., Hauser, J. Y. and Bagg, J. (1985) Polycyclic aromatic hydrocarbons in sediments of the Great Barrier Reef Region, Australia. *Marine Pollution Bulletin* **61** (3), 110–114.

Spies, R. B., Stegeman, J. J., Hinton, D. E., Woodin, B., Smolowitz, R., Okihiro, M. and Shea, D. (1996) Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara Channel. *Aquatic Toxicology* **34**, 195–219.

Stegeman, J. J., and Chevion, M. (1980) Sex differences in cytochrome P-450 and mixed function oxidase activity in gonadally mature trout. *Biochemical Pharmacology* **29**, 553–558.

Stegeman, J. J., Renton, K. W., Woodin, B. R., Zhang, Y.-S. and Addison, R. F. (1990) Experimental and environmental induction of cytochrome P450E in fish from Bermuda waters. *Journal of Experimental Marine Biology and Ecology* **138**, 49–67.

Stegeman, J. J. and Hahn, M. (1994) Biochemistry and molecular biology of monoxygenases; current perspectives on forms, functions and regulation of cytochrome P-450 in aquatic species. In *Aquatic Toxicology*, eds. D. C. Malins and G. K Ostrander, pp. 87–178. CRC Press, Boca Raton, USA.

Stegeman, J. J., Kloepper-Sams, P. J. and Farrington, J. W. (1986) Monooxygenase induction and chlorobiphenyls in the deep-sea fish, *Coryphaenoides armatus. Science* **231**, 1287–1289.

Stegeman, J. J., Woodin, B. R., Singh, H., Oleksiak, M. F. and Celander, M. (1997) Cytochromes P-450 (CYP) in Tropical fishes: catalytic activities, espression of mulitple CYP proteins and high levels of microsomal P-450 in liver of fishes from Bermuda. *Comparative Biochemistry and Physiology* **116C** (1), 61–75.

Van der Oost, R., Keida, H., Opperhuizen, A. and Vermuelen, N. P. E. (1991) Interrelationships between bioaccumulation of organic trace pollutants (PCBs, organochlorine pesticides and PAHs) and MFO-induction in fish. *Comparative Biochemistry and Physiology* **100C** (1/2), 43–47.

Von Westernhagen, H. and Klumpp, D.W. (1995) Xenobiotics in fish from Australian tropical coastal waters, including the Great Barrier Reef. *Marine Pollution Bulletin* **30** (2), 166–169.

Vrolijk, N.H., Targett, N.M., Woodin, B.R., and Stegeman, J.J. (1994) Toxicological and ecological implications of biotransformation enzymes in the tropical teleost *Cheatodon capistatus. Marine Biology* **119**, 151–158.

Wolanski, E., Mazda, Y., King, B. and Gay, S. (1990) Dynamics, flushing and trapping in Hinchinbrook Channel, a giant mangrove swamp, Australia. *Estuarine, Coastal and Shelf Science* **31**, 555–579.

Woodin, B. R., Smolowitz, R. M. and Stegeman, J. J. (1997) Induction of cytochrome P-450 1A in the intertidal fish *Anoplarchus purpurescens* by Prudhoe Bay Crude Oil and environmental induction in fish from Prince William Sound. *Environmental Science and Technology* **31** (4), 1198–1205.

Yellowlees, D. (1991) Land use patterns and nutrient loading of the Great Barrier Reef region. In *Proceedings of the Workshop Held at James Cook University of North Queensland*, November 1990, 234 pp.