The use of biomarkers in barramundi (*Lates calcarifer*) to monitor contaminants in estuaries of Tropical North Queensland

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Summary

In this study water, sediment and barramundi (*Lates calcarifer*) samples were collected from five North Queensland estuaries along a perceived pollution gradient. Water and sediment samples were analysed for trace organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine and organophosphate insecticides and metals. As well, the pollution-induced responses of a suite of seven biochemical parameters (phase I biotransformation enzymes (eg. EROD, P450), fluorescent aromatic compounds (FACs), DNA damage, RNA:DNA ratio and neurotransmission enzymes) and two condition indices (condition factor and hepatosomatic index) were measured in barramundi. The resulting database was subjected to uni- and multi-variate analyses in order to determine the most suitable biomarkers to asses pollution in North Queensland estuaries and to classify the environmental quality of the sites. Principal component analysis (PCA) on the biochemical markers revealed that EROD, EROD/P450, DNA damage and to a lesser extent cholinesterase activity (ChE) and fluorescent aromatic compounds (FACs) were found to be responsive to contaminants in the environment while cytochrome P450, condition factor and the hepatosomatic index were found to be less responsive biomarkers. Of particular significance was the ability of the ChE assay to detect the presence of organophosphate insecticides, compounds that are notoriously difficult to detect in environmental samples analytically. Discriminant analysis (DA) was used to classify the pollution status of the various estuaries. It appears that the best discrimination between the various sites was obtained using DA on the biomarkers; however, further analysis using water quality parameters and levels of organic contaminants in water and sediment produced a similar pattern as found with the biomarkers. This was the first study to employ multiple biomarkers in a resident fish species in Queensland and has demonstrated the utility of applying a multi-biomarker approach in conjunction with traditional analysis of contaminants in providing valuable information in environmental risk assessment.

1. Introduction

This study was carried out adjacent to habitats of The Great Barrier Reef World Heritage Area (GBRWHA) and Wet Tropics Rainforest Area, extremely sensitive and important conservation regions encompassing nationally and internationally significant ecosystems (Williams, 2001; The State of Queensland and Commonwealth of Australia, 2003). The Great Barrier Reef attracts numerous visitors with approximately 6.4 million visits annually, contributing more than AUD $5 billion to the Australian economy and employing more than 63,000 people (Access Economics, 2005). Besides tourism, other key industries within the Great Barrier Reef (GBR) catchment that contribute significantly to the Australian economy are mining, agriculture, fisheries and aquaculture.

Over the last 150 years of European settlement the catchment areas adjacent to the GBRWHA and Wet Tropics Rainforest Area have undergone significant modification, including increased urbanisation and agricultural development. In fact, Gilbert and Brodie (2001) estimated that as much as 80 % of the land in catchments adjacent to these areas has been modified in some way. This has lead to increasing concern about the possibility of contaminants, particularly agricultural compounds, moving into these sensitive ecosystems (Olafson, 1978; Klumpp and von Westernhagen, 1995; Haynes and Johnson, 2000; Haynes et al., 2000; Williams, 2001; Haynes and Brodie, 2004); however, very little information is available about the possible biological implications of this contamination. A number of studies have investigated levels of chemical residues in biological tissues (Olafson, 1978; von Westernhagen and Klumpp, 1995; Russell et al., 1996), while few studies have attempted to use biological indicators to investigate effects of chemical contamination on resident biota (Klumpp and von Westernhagen, 1995; Cavanagh et al., 2000; Codi et al., 2004). This is the first study to investigate possible exposure to and effects of contaminants in a fish using a multibiomarker approach in tropical Australia.

Biomarkers are defined here as biochemical, molecular, physiological or histological indicators that help elucidate the exposure to, or effects of, anthropogenic pollutants within an organism and the magnitude of the response (Depledge et al., 1995; Lam and Gray, 2003), and include responses at the molecular, sub cellular, cellular, tissue and organ level of organization. Responses at higher levels are usually defined as bioindicators or ecological indicators (Lam and Gray, 2003). Biomarkers are considered important tools for providing an integrated measure of exposure to, and effects of, environmental pollutants in the aquatic environment (Peakall, 1994; Lam and Gray, 2003; van der Oost et al., 2003). Measuring biomarkers in animals in situ provides information on the integrated impact of multiple pollution stressors as well as natural environmental stressors including hypoxia, thermal stress, fluctuating food and predation. Biomarkers are particularly valuable as early warning signals of environmental degradation (van Dam et al., 1998; Lam and Gray, 2003) and provide an inexpensive, rapid and highly sensitive means of identifying and evaluating exposure to, and/or effects of, environmental contaminants in complex ecosystems. By selecting a key component in the ecosystem, such as barramundi (*Lates calcarifer*), a top-level predator, and measuring multiple biomarkers including measures of molecular, genetic and physiological impairment along with chemical analysis the ecological relevance of environmental contaminants may be more readily elucidated and thus integrated into environmental management strategies (Brown et al., 2004; Galloway et al., 2004a; Galloway et al., 2004b).

In order to assess whether chemical contaminants were impacting upon these sensitive ecosystems, barramundi were sampled from estuaries of five separate river systems, which represent varying degrees of impact from anthropogenic activities. A multibiomarker approach was used in conjunction with chemical analysis of water and sediment from the five systems to try and characterise the relationship between anthropogenic contamination and

response of resident biota in estuaries along the north Queensland coast. The biomarkers selected for this study are classified into three groups: 1) biomarkers of general condition; hepatosomatic index (HSI) , condition factor (K) and RNA:DNA ratio, all of which are influenced by generic stressors; 2) biomarkers of exposure to specific contaminant classes; organophosphate or carbamate insecticides (cholinesterase activity), petroleum hydrocarbons (fluorescent aromatic compounds, FACs), induction of the cytochrome P450 monooxygenase system (EROD) and 3) a biomarker for genotoxins (Fast Micromethod®). These biomarkers were selected primarily with regard to the potential contaminants in each estuary, ease of use, low cost and environmental relevance.

2. Materials and Methods

2.1 Sampling sites

Five rivers were selected on the basis of human activities within these catchments. Land use patterns and indicative pesticide usage is summarised in Table 1. Both the Herbert and Johnstone catchments are heavily utilised for agriculture including cattle grazing, sugarcane production and horticulture. Sugarcane is of particular interest as it is mainly grown on the coastal floodplains within 50 km of the coast and is heavily reliant upon herbicides and insecticides (Hamilton and Haydon, 1996). Both catchments have been heavily modified and have population centres on the main rivers; hence both rivers receive discharges from urban runoff and sewerage. In addition the Johnstone River receives industrial effluent from a sugar mill and a commercial port. The Endeavour River is situated at Cooktown, which has a small population and with limited agricultural impact on the catchment. Limited marine traffic, which services the tourism and fisheries industries, uses the Endeavour River as a base or port-of-call. Both the Lockhart and Pascoe Rivers are situated on Cape York Peninsula, which is one of the least disturbed regions of Australia. Due to its isolation, few people inhabit the area so it is undeveloped with no major population centres or industry.

Table 1: Summary of human activities within the catchments of the five rivers sampled. *Estimated annual amounts used.

	Herbert River	**Johnstone River**	**Endeavour River**	**Lockhart River**	**Pascoe River**
Population [a,b]	8778	13428	1344	<1000	<500
Area (km^2)[c]	9843	2325	2104	2883	4179
Area under grazing (km^2)[a,c]	7330	493	1768	574	2549
Area under sugar (km^2)[a,c]	691	394	0	0	0
Area under horticulture (km^2)[a,c]	35	44	0	0	0
Atrazine (kg active ingredient yr^{-1})[d]*	33601	25284	0	0	0
Diuron (kg active ingredient yr^{-1})[d]*	16618	17353	0	0	0
2-4 D (kg active ingredient yr^{-1})[d]*	28068	14938	0	0	0
Chlorpyrifos (kg active ingredient yr^{-1})[d]*	3084	6313	0	—	—
Methoxy-aethyl mercury chloride MEMC (kg active ingredient yr^{-1})[d*]	397	251	0	—	—

[a] GBRMPA, 2001; [b] http://www.mcmc.qld.gov.au/community/search/lockhart.php; [c] Furnas, 2003; [d] Hamilton & Hayden 1996.

2.2 Study species

Barramundi, *Lates calcarifer* (Bloch), were selected as an indicator species for this study. It is a large predatory fish belonging to the Centropomidae family with a wide distribution throughout Asia and through to the tropical and semitropical waters of northern Australia where they are relatively abundant (Davis and Kirkwood, 1984; Grey, 1987; Russell and Garrett, 1988; Keenan, 1994). Their biology is well known due to their importance as a commercial and recreational fishery. They are catadromous, with adults spawning in inshore coastal waters and estuaries (Garrett, 1987; Russell and Garrett, 1988) before juveniles move to occupy temporary tidal habitats, estuaries, mangrove flats or swamps (Russell and Garrett, 1983; Russell and Garrett, 1988) where they remain until they reach sexual maturity at about 3-4 years of age (55-70 cm total length) (Davis, 1982). Developing gonads are first recognisable in males at approximately 250 – 350 mm and they are sexually mature after about 3 years of age (500 – 600 mm) (Davis, 1982). The vast majority of barramundi start life as males and then undergo sexual inversion to become functional females at around 6 yrs of age (650 – 750 mm). Along the north east Queensland coast maturation in gonads generally commences in August/September and is thought to be triggered by increasing water temperatures and spawning finishes in February (Grey, 1987). Barramundi were selected as a suitable candidate for the biological assessment of these catchments because they are commercially important, are abundant in all of the sampling locations, relatively easy to catch, have been found to be suitability sensitive to chemical contaminants and do well in laboratory studies (Codi et al., 2004).

2.3 Sample collections and preparation

Barramundi were collected during August 2002 from each of five rivers; Herbert River [18° 31' S, 146° 20' E], Johnstone River [17° 30' S, 146° 04' E], Endeavour River [14° 32' S, 145° 14' E], Lockhart River [12° 52' S, 143° 21' E] and Pascoe River [12° 29' S 143° 16' E]. Locations of these rivers are shown in Figure 1. Fish were captured from small boats by means of line and hook and transported alive back to the mother ship in tanks of ambient water. Fish were sacrificed by cervical dislocation, and liver tissue (from the left lobe) was excised immediately, snap-frozen in liquid nitrogen and stored at -80 °C for later analysis of EROD activity, cytochrome P450, genetic damage and RNA:DNA ratio. Muscle tissue (from just anterior to and below the dorsal fin) was sampled for cholinesterase activity (ChE). Bile was collected from the gall bladder using a hypodermic syringe, snap-frozen in liquid nitrogen for later analysis of fluorescent aromatic compounds (FACs). Fork length (TL mm), total and gutted fish weight (g), gut, gonad and liver weight (g) were recorded for each fish.

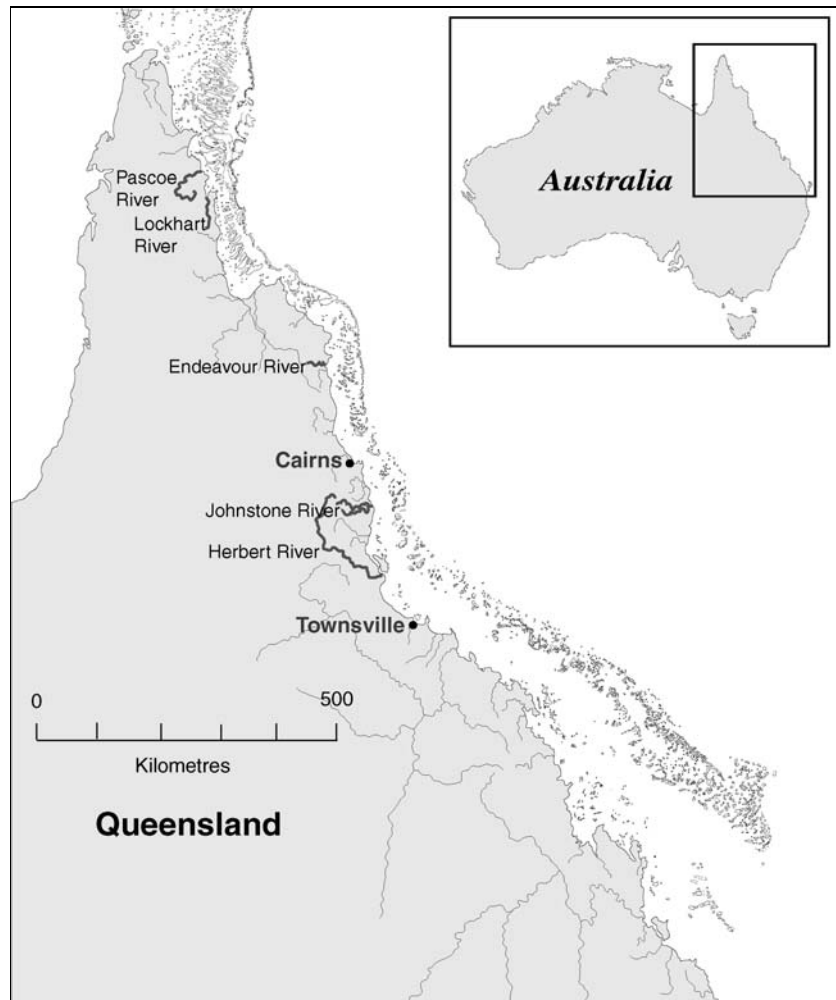

Figure 1: Sampling locations along the northern Queensland coast.

2.4 Biomarkers

2.4.1 Indices

Condition factor (K) was calculated according to Bolger and Connolly (1989):

$$K = S_w/L^3$$

where S_w was the somatic weight (total weight of the fish, less gonad and intestines in grams) and L was total fish length in millimetres. Hepatosomatic index (HSI) was calculated according to Slooff et al. (1983):

$$HSI = (L_w/S_w) \times 100$$

where L_w represents the total liver weight in grams and S_w is the somatic weight as given above.

2.4.2 RNA:DNA

Samples were stored at -80 °C and not allowed to thaw at any time. Briefly, frozen fish liver (~50-100 mg) was ground in a mortar and pestle under liquid nitrogen as described by Zahn et al. (1996) and homogenised in 4 mL of TE buffer (Tris-EDTA (TE) buffer [10 mM Tris-HCl, 1 mM EDTA, pH 7.5] with 1 % sarcosyl). A blank control was also prepared that contained only the extraction buffer and was treated in the same manner as the samples. The samples and blanks were then sonicated in an ice bath for 60 s. After sonication the samples and blanks were centrifuged for 3 min at 1200 × *g* and 150 µL of the supernatant was taken and placed in a deep well plate along with 1350 µL of TE buffer and shaken thoroughly.

Methods for the determination of RNA and DNA are modified from those of Kyle et al. (2003). Briefly, three identical black 96-well microplates were created by adding 75 µL of nucleic acid standards (0-1.5 µg mL^{-1} for DNA and RNA), control homogenates or sample to each plate. Plate 1 had 15 µL of TE buffer and 75 µL of RiboGreen® solution added. Plate 2 had 7.5 µL of TE buffer and 7.5 µL of RNase, which was allowed to incubate at room temperature for 40 min before the addition of 75 µL of RiboGreen®. Plate 3 had 7.5 µL of RNase and 7.5 µL of DNase added and allowed to incubate at room temperature for 90 min before the addition of 75 µL of RiboGreen®. Once each microplate was prepared it was placed into a microplate reader (BioTek Synergy HT) at 25 °C and gently shaken before being read at excitation/emission wavelengths of 485/528 nm, respectively. Fluorescence due to RNA was calculated by subtracting the fluorescence of Plate 2 from Plate 1. Fluorescence due to DNA was calculated by subtracting the fluorescence of Plate 3 from Plate 2. Concentrations of RNA and DNA were then calculated based on the standard curves from each plate.

2.4.3 Ethoxyresorufin O-deethylase (EROD) analysis

EROD activity was determined fluorospectrophotometrically based on the methods of Burke and Mayer (1974) and as further described in Krüner and von Westernhagen (1999). Briefly, liver tissue was thawed on ice, weighed individually and homogenised 1:4 w/v in 0.1 M phosphate buffer (pH 7.4) with a Potter-Elvehjem homogeniser. Homogenates were then centrifuged at 10,000 × *g* for 20 min at 4 °C. The resulting pellet was discarded and the supernatant was centrifuged at 100,000 × *g* for 60 min at 4 °C. The supernatant was discarded and the mitochondrial pellet was resuspended in 2 mL of 0.1 M phosphate buffer (pH 7.4), containing 20 % glycerol. Resuspended microsomes were stored in liquid nitrogen for subsequent analysis.

To a 1 cm glass quartz cuvette 1865 µL of 0.1 M phosphate buffer (pH 7.4), 75 µL of 1.25 µM 7-ethoxyresorufin, 50 µL sample and 10 µL of 1.0 mM NADPH were added. The enzyme kinetics were monitored on a Hitachi F-4000 fluorescence spectrophotometer at excitation/emission wavelengths of 530/585 nm respectively, for a period of two minutes. The measured fluorescence corresponded to the amount of resorufin produced by the enzymatic reaction. This value was equivalent to the crude mixed function oxidase (MFO) activity of the sample measured as production of resorufin per minute per mg protein (pmol min^{-1} mg $prot.^{-1}$). The purity of the resorufin standard was determined as described in Stagg and McIntosh (1998).

2.4.4 Cytochrome P-450 Content dithionite difference

Total cytochrome P-450 content was analysed in the microsomal preparation produced above by the dithionite reduced difference spectrum of carbon monoxide bubbled samples, according to the method of Omura and Sato (1964) and Matsubara et al. (1976) with modification as described by Rutten et al. (1987) on a Shimadzu UV 1601 double beam spectrophotometer. Briefly, 400 µL of the microsomal suspension was added to 3.6 mL of 0.1 M phosphate buffer (pH 7.4) and bubbled slowly with carbon monoxide for 30 s. The sample was then divided equally between two cuvettes and allowed to sit for two minutes. The

baseline was recorded and the sample was then reduced by the addition of 10 µL sodium dithionite (final concentration, 4.58 mM). After three minutes the dithionite difference spectrum was recorded at wavelengths between 400-500 nm. The concentration of cytochrome P-450 was calculated from the difference in absorbance at 450 and 490 nm, using an extinction coefficient (ε) of 104 mM^{-1} cm^{-1} (Matsubara et al., 1976). Results were expressed as nmol mg $protein^{-1}$.

2.4.5 Cholinesterase activity

Cholinesterase (ChE) activity was determined spectrophotometrically by the method of Ellman et al. (1961), modified for microplate reading as described in Bocquené and Galgani (1998). Briefly, frozen muscle tissue (1.5 to 2.5 g) was thawed on ice and was then homogenised 1:5 w/v in 0.02 M phosphate buffer (pH 7.0) containing 0.1% Triton X 100 using a Heidolph Diax 900 homogeniser set on 4. The homogenate was then centrifuged at 10,000 × *g* for 20 min at 4 °C and an aliquot of the supernatant used in the ChE and protein assays.

To each well of a microplate 300 µL of 0.02 M phosphate buffer (pH 7.0), 20 µL of dithiobisnitrobenzoic acid (DTNB 0.01 M) and 10 µL of sample were added successively. Blanks were run containing no sample and 310 µL of phosphate buffer to correct for non-enzymatic hydrolysis. After 5 min incubation at room temperature 10 µL of acetylthiocholine iodide (ACTC 0.1 M) was added to start the reaction. The enzyme kinetics were monitored on a microplate reader (Tecan Spectra II) at 405 nm every 10 s for 2 min. Enzyme activity was expressed as the amount of enzyme which catalyses the hydrolysis of 1 µmole of acetylcholine per min per mg protein (µmol ACTC min^{-1} mg $protein^{-1}$).

Protein concentrations of the supernatant were determined spectrophotometrically using the Bio-Rad DC protein assay kit (Richmond, CA, USA) with bovine serum albumin (BSA) as standard, based on the method of Lowry et al. (1951).

2.4.6 Fast Micromethod®

The Fast Micromethod® assay was performed according to Batel et al. (1999) based on the unwinding of cellular DNA under alkaline conditions. Briefly, frozen fish liver (~50-100 mg) was homogenised in 4 mL of TE buffer (1 mM EDTA, 10 mM Tris, 5 % dimethylsulfoxide, and 1 % N-lauroylsarcosine) in a mortar and pestle under liquid nitrogen as described by Zahn et al. (1996). The homogenate was diluted 10-fold with TE buffer (pH 7.4). First, a 25 µL aliquot of sample was distributed into the wells of a black 96-well microplate, followed by the addition of a 25 µL aliquot of lysing solution (9 M urea, 0.1 % N-lauroylsarcosine, 0.2 M, EDTA) containing 20 µL mL^{-1} of PicoGreen® solution. Lysis of the sample was allowed to occur on ice in the dark for 1 h. DNA unwinding was started with the addition of 250 µL of 0.025 M NaOH (pH 12.4) to each sample. Fluorescence was measured at 485 nm excitation and 528 nm emission every 60 s for 1 h using a BioTek Synergy HT plate reader.

Results were expressed as strand scission factors (SSFs), according to the equation: SSF = log (% dsDNA in sample / % dsDNA in control). This value was calculated in relation to control values at 0-time denaturation after correction for blank readings and unwinding was followed for 20 min. Thus a SSF of 0 indicates no additional strand breaks and alkaline-labile sites relative to controls, while negative SSF values are indicative of increasing strand breaks.

2.4.7 Fluorescent Aromatic Compounds (FACs)

Fluorescent aromatic compounds (FACs) in bile were measured in barramundi by high performance liquid chromatography using fluorescence detection as described in detail in Codi King et al. (2005). The bile sample was injected and the eluant was monitored at

excitation and emission wavelengths specific for naphthalene and its metabolites (290/336 nm). Peak areas were summed and converted to naphthalene equivalents (wet weight) using a calibration curve for naphthalene. Bile protein was determined using the Bio-Rad DC protein assay kit (Richmond, CA, USA), based on the method of Lowry et al. (1951). All FAC results were normalised to protein concentration to account for variation due to sampling and feeding status and expressed as µg naphthalene equivalents per mg protein.

2.5 Water sampling and analysis

At each sampling location, general physico-chemical parameters were measured including pH, salinity (ppt), temperature (°C) (TPS Model WP81 portable meter) and dissolved oxygen (mg L^{-1}; YSI Model 51A oxygen meter, Yellow Springs Instrument Co.). Between five and seven water samples were collected from each estuary. Surface water (<1 m) was sampled for analysis of polycyclic aromatic hydrocarbons (PAHs).

A 1 L sample of surface water was extracted for PAHs with 100 mL of dichloromethane. The extraction was repeated a second time and extracts were combined and concentrated. Following extraction, the sample was cleaned up using gel permeation chromatography (GPC) using a Waters Envirogel. The extract was concentrated to a final volume of 100 µL and analysed for 16 priority pollutant PAHs: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chyrsene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene. Extracts were analysed on a Shimadzu GC17 gas chromatograph (GC) fitted with a QP5050A mass spectrometer (MS) equipped with a DB-5 column for PAHs using helium as the carrier gas.

2.6 Sediment sampling and analysis

Sediments were collected using a 15 cm^{-3} scissor grab. Three grab samples were combined to form a composite sample, which was then mixed thoroughly in a stainless steel bowl, sub-sampled and frozen at -20 °C until analysed. Sediments were analysed for suite of chemicals which included organochlorine and organophosphorus insecticides, phenoxy herbicides, PAHs, polychlorinated biphenyls (PCBs) and total heavy metal residues.

All PAH, PCB, pesticide and 2,4-dichlorophenoxy acetic acid (2,4-D) sediment extracts were prepared and analysed by Queensland Health State Service (QHSS) laboratory (Coopers Plains, Qld, Australia) using method 16313 (AOAC, 1990). For PAH and PCB samples a 50 g aliquot of wet sediment was mixed with hydromatrix (diatomaceous earth) to form a free flowing powder which was then extracted using Dionex ASE100 (Accelerated Solvent Extraction). The sediments were extracted with a solvent mixture consisting of a 50:50 ratio of dichloromethane:acetone (v/v). The sample was heated to 125 °C with a static cycle of 5 min. For pesticides a 50 g aliquot of wet sediment was extracted with a mixture of 50:50 acetone:hexane (v/v) using a flat-bed automatic shaker. The solvent extract was concentrated on a rotary evaporator and solvent swapped to dichloromethane. For 2,4-D, 50 g of wet sediment was mixed with 100 mL of 0.1 M Sodium Hydroxide (NaOH) and shaken for 4 h. The extract was adjusted to pH 2 and back extracted into diethyl ether. The solvent extract was concentrated and methylated before being analysed. All sediment extracts were cleaned up using gel permeation chromatography (GPC) using a Waters Envirogel.

For PAHs, PCBs and all pesticides sediment extracts were concentrated to a final volume of 100 µL. PAHs, PCBs and 2,4-D were analysed on a Shimadzu GC17 gas chromatograph fitted with a QP5050A mass detector (GC/MS) equipped with a DB-5 column and HT8 column using helium as the carrier gas. Diuron and atrazine were analysed on a AB/Sciex API 300 mass spectrometer equipped with heated Nebuliser on a Alltima C_{18} column

(Waters, 150 x 4.6 mm 5 µM) with a methanol/0.005 M ammonium acetate mobile phase. Quantification was done on selected ions for each individual component analysed and concentrations were calculated using the internal standard method. A separate aliquot of wet sediment (~10 g) was dried overnight in a laboratory oven at 105 °C then weighed gravimetrically and the percent moisture content was determined. Results are reported on a dry weight basis.

Sediment samples were also analysed for Fe, Mn, Co, Ni, Cu, Zn, and Pb using an inductively coupled plasma atomic emission spectrometer (Varian Liberty ICP-AES) following a modified acidification procedure (Loring and Rantala, 1992). Cadmium was analysed separately on a Zeeman atomic absorption spectrometer (AAS). Briefly, 0.5 g of sediment, dried at 80 °C for 24 h and ground, was weighed into a 50 mL digestion tube, digested with HNO_3 and $HClO_4$ at 120 °C for 3 h, then refluxed at 180 °C for 3 h, and made up to 50 mL in a volumetric tube. The resulting complex was analysed sequentially. Analytical performance was monitored with standard reference materials NRCC MESS-1 (marine sediment) and MESS-3 (marine sediment). Values were always within the certified range. Analytical precision was ±3 %.

2.7 Statistical analysis

For the biomarker data, fish biometrics, water quality parameters and physico-chemical data, differences between means for each river were examined by one factor analysis of variance (ANOVA). Data were tested for homogeneity of variances using the Brown-Forsythe test and when necessary data were log transformed. Results were considered significantly different if $p < 0.05$. If significantly different *post hoc* comparisons of the means for significant factors in the ANOVAs were carried out using the Tukey significant-difference for unequal N multiple-comparisons test. Data are reported as untransformed means ± standard error unless otherwise stated.

Principal components analyses (PCA) was performed on all biomarkers in fish, organic contaminant in sediment and waters and metals in sediments using location as the grouping variable. Data were normalised by taking their z scores. Discriminant analysis was carried out on the same data sets and tree diagrams were created using single linkage on squared Mahalanobis distances. Statistical analysis were performed using either S-Plus or Statistica 6.0, StatSoft Inc., Tulsa USA. All results in the text are given as the mean ± standard error unless otherwise indicated and plots are all of untransformed data.

3. Results

3.1 Fish biometrics

The main characteristics of the fish are given in Table 2. No significant differences were observed in terms of total weight, total length or liver weight between the five river systems. There was no significant difference in the condition factor (K) or the hepatosomatic index (HSI) in the fish from the five different river systems. In addition, all fish were either immature or males caught prior to the spawning season. Thus any differences measured in the biomarkers were not a consequence of sex or size, and these factors can be excluded from the analysis.

Table 2: Total length, weight, liver weight, condition factor (K) and hepatosomatic index (HSI) of Lates calcarifer from the five study rivers (means±s.e.).

	Johnstone River (n = 14)	Herbert River (n = 10)	Endeavour River (n = 9)	Pascoe River (n = 15)	Lockhart River (n = 15)
Total Length (mm)	456±20	465±17	459±32	505±16	472±12
Weight (g)	1062±143	1091±118	1139±250	1492±135	1239±96
Liver Weight (g)	10.17±0.99	11.84±1.17	14.13±3.70	13.03±1.16	11.33±0.77
K	1.04±0.04	1.05±0.03	1.06±0.04	1.12±0.02	1.15±0.04
HSI	1.07±0.08	1.10±0.06	1.10±0.09	0.92±0.07	0.94±0.05

3.2 Biomarker analysis

The results for ChE, EROD, cytochrome P-450, EROD turnover rates (EROD/P450), DNA damage, FACs and RNA:DNA ratios in barramundi are shown in Table 3 and Figure 2. There was a significant difference in six of the seven biomarker responses in the fish sampled from the five estuaries studied (Table 3).

The ChE response in barramundi muscle tissue shows significant differences between the five rivers (Table 3, Fig. 2A). There was significant inhibition of ChE in fish from the Herbert River (0.20±0.01 μmol ACTC min mg protein^{-1}) when compared with those from the Lockhart (0.38±0.02 μmol ACTC min mg protein^{-1}), Pascoe (0.34±0.02 μmol ACTC min mg protein^{-1}) and Endeavour Rivers (0.34±0.02 μmol ACTC min mg protein^{-1}), while the Johnstone River (0.25±0.04 μmol ACTC min mg protein^{-1}) showed significant inhibition when compared to the Lockhart River.

EROD activity was significantly different in fish from the five rivers (Table 3, Fig. 2B). The Johnstone River fish had at least five times higher EROD activity (34.6±4.11 pmol min mg protein^{-1}) than that of fish from the Lockhart (4.76±1.63 pmol min mg protein^{-1}), Pascoe (3.20±1.27 pmol min mg protein^{-1}) and Endeavour (6.39±1.70 pmol min mg protein^{-1}) Rivers while EROD activity in fish from the Herbert River (16.9±4.74 pmol min mg protein^{-1}) was significantly higher than in those from the Lockhart and Pascoe Rivers (Fig. 2B). The EROD turnover rates show a similar pattern to the EROD activity (Fig. 2C), although there was no significant difference seen in the expression of cytochrome P-450 (Table 3).

FACs were present in bile of all fish collected (Fig. 2D), and levels of these differered significantly between the five rivers sampled (Table 3). Barramundi from the Johnstone River (45.1±4.24 μg mg protein^{-1}) had significantly higher levels of FACs than fish from the Herbert

(14.0±2.42 μg mg protein^{-1}), Pascoe (20.7±3.31 μg mg protein^{-1}), Lockhart (28.1±2.97 μg mg protein^{-1}) and Endeavour (31.0±5.46 μg mg protein^{-1}) Rivers.

There was a significant difference in DNA damage in liver of barramundi from different rivers as measured by the Fast Micromethod® (Table 3). Strand scission factors (SSFs) for fish from the Johnstone River (0.37±0.02 SSFs) were higher than those for the fish from the Herbert (0.29±0.02 SSFs), Pascoe (0.22±0.01 SSFs), Lockhart (0.22±0.01 SSFs) and Endeavour (0.28±0.03 SSFs) Rivers (Fig. 2E)

RNA:DNA ratios were significantly different in fish caught from the five different river systems (Table 3 and Fig. 2F). Fish from the Johnstone River had the lowest RNA:DNA ratio values (2.4±0.17) and this level was considerably lower than fish from the Herbert (3.1±0.11), Pascoe (3.3±0.13), Lockhart (3.6±0.17) and Endeavour (3.0±0.21) Rivers.

Table 3: ANOVA outputs testing for differences in biomarker response in barramundi (*Lates calcarifer*) sampled from the five river systems.

	df	*MS*	*F*	*p*
ChE	58	0.0098	6.6977	**<0.0002**
EROD	58	1.5074	27.6357	**<0.0001**
P450	58	0.0000	1.8257	0.1362
EROD/P450	58	0.0072	28.1101	**<0.0001**
DNA damage	58	0.0036	15.1473	**<0.0001**
FACs	42	150.2622	9.2505	**<0.0001**
RNA:DNA	58	0.3359	7.6619	**<0.0001**

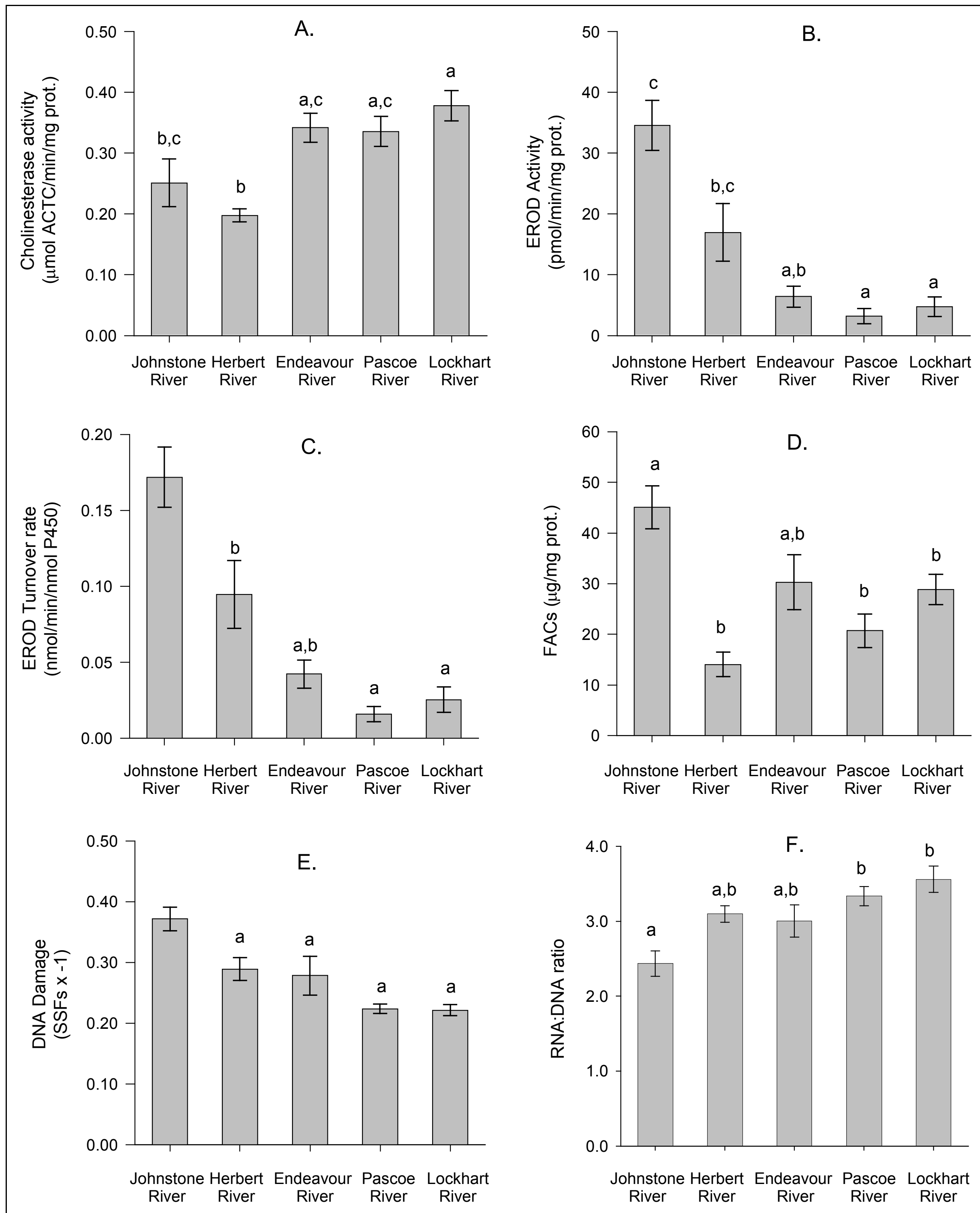

Figure 2: Biomarkers measured in barramundi (*Lates calcarifer*) from five river systems in North Queensland (mean±s.e.); (A) ChE activity, (B) EROD activity, (C) EROD turnover rate, (D) Fluorescent aromatic compounds, (E) DNA damage and (F) RNA:DNA ratios. Bars without a common letter are significantly different (p ≤ 0.05, Tukey HSD test).

3.3 Water and sediment analysis

Table 4 shows the levels of contaminants found within sediment and water sampled from the five estuaries. Low molecular weight PAHs were consistently found in all water samples from the Johnstone and Herbert Rivers and mainly comprised of phenanthrene and naphthalene. No other contaminants were found in water. Higher molecular weight PAHs were found in sediments from the Johnstone, Herbert, Endeavour and Pascoe Rivers, although their distribution was patchy. In both the Johnstone and Endeavour Rivers, the only PAH to be detected was benzo(*a*)pyrene in two of five samples from the Johnstone River and in one of the five samples from the Endeavour River. Diuron was the only agrichemical detected in any of the sediment samples and there was a significant difference between the estuaries sampled ($F_{(4,24)} = 7.46$, $p < 0.005$). Diuron was found in all sediment samples from the Johnstone (n=5) and Herbert Rivers (n=5) in the range of 0.3 – 3.9 and 0.1 – 4.1 µg kg^{-1} dry weight respectively. Diuron was not detected in sediment samples from any of the other estuaries.

Table 4: Chemical contaminants found in sediment and water from the five rivers sampled.

	Detection Limits	**Johnstone River**	**Herbert River**	**Endeavour River**	**Pascoe River**	**Lockhart River**
Sediments						
Ni (mg kg^{-1})	2.00	58.8±17.5	10.0±5.03	3.80±3.80	0.00±0.00	5.00±1.55
Cu (mg kg^{-1})	2.00	23.2±5.34	9.20±4.35	4.60±1.63	1.60±0.678	6.25±1.05
Zn (mg kg^{-1})	0.500	77.8±15.4	44.6±20.3	13.6±7.48	7.00±1.52	34.8±7.27
Pb (mg kg^{-1})	5.00	11.0±2.82	14.8±7.93	5.40±3.60	2.40±1.50	15.1±3.72
Cd (µg kg^{-1})	5.00	36.7±12.3	12.0±9.70	22.0±6.63	24.0±2.45	13.8±4.61
Cr (mg kg^{-1})	5.00	77.5±13.8	13.2±7.17	13.6±4.71	2.40±1.47	15.7±3.59
As (mg kg^{-1})	0.250	11.0±2.76	9.64±4.60	2.26±1.09	2.18±0.328	11.5±2.73
Hg (µg kg^{-1})	1.00	31.5±10.7	15.4±7.38	10.8±3.93	6.20±0.970	20.5±4.38
Diuron (µg kg^{-1})	1.00	2.23±0.504	1.38±0.796	ND	ND	ND
Σ PAHs (µg kg^{-1})	2.00	187±171	11.0±6.45	37.4±37.4	6.40±3.87	ND
Water						
Σ PAHs (µg L^{-1})	2.00	10.3±6.43	2.48±1.34	ND	ND	ND

ND- represents values below the detection limit or non-detected.

3.4 Multivariate analysis

The PCA, looking at all biomarker responses in barramundi from the five estuaries (Fig. 3A) shows that fish from the Johnstone River can be characterised by having higher rates of DNA damage, EROD activity, EROD turnover and ChE inhibition and lower RNA:DNA ratios in comparison to fish from other rivers. The Herbert River has similar characteristics to the Johnstone River in terms of biomarker response. These results are in contrast to the Pascoe and Lockhart Rivers which are grouped together and are characterised by having lower rates of DNA damage, EROD activity, EROD turnover rates, lower rates of ChE inhibition and higher RNA:DNA ratios in comparison to the Herbert and Johnstone Rivers. The Endeavour River is intermediate to these two groups. These patterns are clearly reflected in the tree diagram resulting from performing a cluster analysis on the biomarker responses in barramundi (Fig. 3B) which place the Johnstone and Herbert Rivers in a discrete group and the Pascoe and Lockhart Rivers in another discrete group with the Endeavour River in the middle though more closely linked to this latter group.

The next stage of analysis included assessing the relationship between the organic contaminants and the five river systems and metals sampled from the five locations using PCA (Fig. 3C & E). The PCA for organic contaminants shows that the Johnstone River was characterised by higher levels of PAHs in water and diuron in sediments than the Endeavour, Lockhart and Pascoe Rivers. The pattern is similar to the one shown by the Herbert River (Fig. 3C). These results are not surprising given that these were the only two rivers where these chemical contaminants were found (Table 4). PAHs in the sediment had less of an effect on the PCA due to their being found in samples from all rivers apart from the Lockhart River. The tree diagram of the resulting cluster analysis (Fig. 3D) shows the Lockhart and Pascoe Rivers grouped together, and close to the Endeavour River with the Herbert River between it and the Johnstone River which is apart from the other rivers.

The PCA of the relationship between metal contamination in the five locations (Fig. 3E) shows that the overall pattern is heavily influenced by a small number of very large values. These are mainly high levels of cadmium, copper, nickel, zinc and chromium occurring in samples from the Johnstone River (Table 4). The corresponding tree diagram of the resulting cluster analysis of metal contamination illustrates this point where the Lockhart and Pascoe Rivers are once again grouped together, and both the Endeavour and Herbert Rivers are grouped, but closely linked to the other group. The Johnstone River as a site with relatively high heavy metal contamination, sits on its own a considerable distance from the other groups.

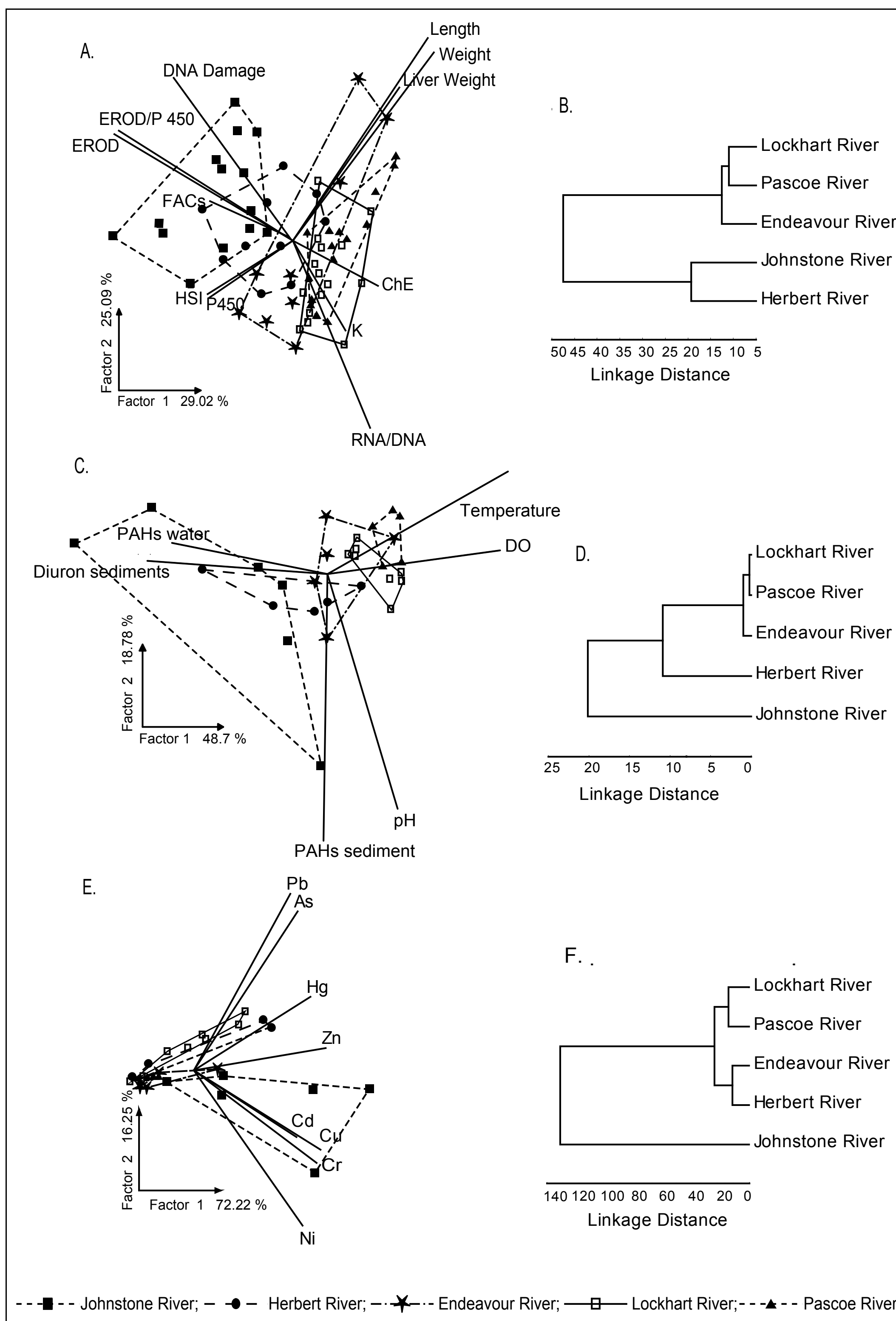

Figure 3: Principal components analysis and dendograms (cluster analysis; single linkage on squared Mahalanobis distances); (A & B) biomarker responses, (C & D) organic contaminants, and (E & F) metal contamination.

4. Discussion

In the present study a multi-biomarker approach, using barramundi (*Lates calcarifer*), was combined with chemical analysis of sediments and water to evaluate differences in environmental pollution exposure and sublethal effects in biota in five estuaries along the North Queensland coast, providing a preliminary evaluation of ecosystem condition. The five estuaries were chosen to represent varying degrees of anthropogenic activities and sampled at the same time of the year to minimise any seasonal effects. This study showed that fish from the Johnstone River and to a lesser extent the Herbert River were exposed to a variety of different classes of contaminants which are indicated by the response of the various biomarkers measured. The biomarker results correlated to levels of contaminants in water and sediment though the measurements in these compartments were patchy, possibly illustrating the limitations of traditional "spot" chemical analysis.

Three indices of general condition were investigated in barramundi; condition factor (K), hepatosomatic index (HIS) and RNA:DNA ratio in liver tissue. K, based on the length-weight relationship of a fish has often been used as an indication of general fitness of a fish (Bagenal and Tesch, 1978; Bolger and Connolly, 1989) as well as to investigate the effects of contaminants (eg. Laroche et al., 2002; Bervoets and Blust, 2003; Pyle et al., 2005). In this study no significant difference was found in K between the five estuaries studied indicating that generally the fish were in relatively good condition. HSI is a measure of energetic reserves of the liver and metabolic activity, and has been shown to increase in response to chemical contamination (Slooff et al., 1983). Enlargement of the liver is due to either an increase in cell size (hypertrophy) or an increase in cell number (hyperplasia) (van der Oost et al., 2003). There was no statistically significant difference in HSI in the fish sampled from the five estuaries in this study. The RNA:DNA ratio, which provides an estimate of nutritional condition and recent (days) protein synthesis (Buckley, 1984; García et al., 1998; Buckley et al., 1999) was lower in fish from the Johnstone River compared with fish from the Lockhart and Pascoe Rivers. Though pollution impacts can potentially affect K (Hodson et al., 1992; Pyle et al., 2005), HSI (Billiard and Khan, 2003; Khan, 2006) and RNA:DNA ratios (De Boeck et al., 1997), none of these methods are particularly sensitive to contaminants and are generally more sensitive to other parameters such as habitat, food quality and food availability (Lobon-Cervia et al., 1991; Wu et al., 2003).

The cytochrome P450 enzyme system, estimated by the enzymatic activity of 7-ethoxyresorufin *O*-deethylase (EROD), is known to play a major role in the biotransformation of environmentally relevant xenobiotics. EROD activity has been shown to be an extremely sensitive biomarker for exposure to organic contaminants, particularly planar halogenated hydrocarbons (PHHs), polycyclic aromatic hydrocarbons (PAHs), polychlorobyphenols (PCBs), pesticides, dioxins and furans (Holdway et al., 1995; Stegeman et al., 1997; Whyte et al., 2000). It has been extensively used to monitor a wide range of contaminant sources including urban run-off and industrial outfalls (e.g. Sole et al., 2002; Gravato and Santos, 2003), paper pulp mills (e.g. Khan and Payne, 2002), and petroleum production (e.g. Davies et al., 1984; Stagg et al., 1995). There was a significant induction of EROD activity in fish from both the Johnstone and Herbert Rivers with a five-fold and three-fold induction relative to the fish collected from the other systems. The elevated induction is similar to a previous study that showed a two to three-fold EROD induction in barramundi from the Johnstone River in comparison to another river on Cape York Peninsula, the Olive River (Codi et al., 2004). Higher levels of EROD induction in barramundi from the Johnstone River, and to a lesser extent the Herbert River, in comparison to the other rivers appear to reflect the land use patterns in the catchments of the different rivers as shown in Table 1, despite the relatively low population density of one to five people per km^2. In addition to these land uses, the Johnstone River is serviced by a small port facility for a commercial fishing fleet which includes a small dry dock for ship repair and maintenance, and a sewerage treatment plant

on the banks of Ninds Creek that flows into the main river. Chemical analysis reflected these different land use practices showing higher levels of PAHs in both the sediment and water and higher levels of diuron in sediment in both of these rivers. Previous studies have confirmed detectable concentrations of lindane, dieldrin and DDE in sediments off the Johnstone River while failing to detect any of these contaminants in any rivers sampled on Cape York Peninsular (Haynes et al., 2000).

EROD induction in fish has commonly been used as an indicator of exposure to PHHs and PAHs (Goksøyr and Förlin, 1992; Pinkney et al., 2004; Lee and Anderson, 2005) and some work has been conducted in barramundi (Codi et al., 2004). Barramundi were exposed to the model PAH inducer β-naphthaflavone (β-NF) by intra-peritoneal injection, showing that EROD induction in barramundi was dose-dependent and occurred rapidly, within 4 h, at concentrations ranging from 5 – 50 mg β-NF kg^{-1} (Codi et al., 2004). At 5 mg β-NF kg^{-1} maximum induction after 4 h was 88.6±51.9 pmol min^{-1} mg $protein^{-1}$ which is approximately twice the EROD induction found in fish sampled from the Johnstone River. These data support the hypothesis that barramundi living in this catchment are being exposed to a moderate level of induction based upon the scaling used by Whyte et al. (2000).

There was no difference in total cytochrome P450 (cyt P450) levels in fish across all sites. This finding is consistent with a previous laboratory study looking at cyt P450 induction in barramundi which found no clear dose-dependent response between β-NF dosage and total cyt P450 (Codi et al., 2004). A recent review by van der Oost et al. (2003) showed that in over 50% of field and laboratory studies a strong and significant increase in total cyt P450 was observed; however, despite these finding, total cyt P450, measured spectrophotometrically (Matsubara et al., 1976), is considered of limited value as a biomarker in fish as it is representative of all forms of cyt P450 and does not provide information on the identity or function of the protein (Stegeman and Hahn, 1994; van der Oost et al., 2003). Despite these limitations, it has been recommended as a rapid and inexpensive method of determining the integrity of microsomal preparations as peaks at 420 nm are indicative of degradation (Stegeman and Hahn, 1994; van der Oost et al., 1996).

EROD turnover rates, or EROD activity normalised to cytochrome P450, has previously been used to provide additional information on the induction of cytochrome P450 isozymes. Gallagher and Di Giulio (1989), in a study looking at the effects of complex waste mixtures in brown bullheads (*Ictalurus nebulosus*), found significantly higher EROD turnover rates in fish from polluted sites in comparison to the reference site, yet found no differences in EROD activity. In the present study EROD turnover rates were just a reflection of EROD activity which is consistent with previous laboratory studies using barramundi (Codi et al., 2004).

Fish have been shown to readily take up petroleum hydrocarbons, including PAHs, from water, sediment and food (Cohen et al*.,* 2001), that upon metabolism have been implicated in the prevalence of liver neoplasia in fish (Anulacion et al., 1998; Myers et al., 1998). However, measuring PAHs in body tissues of fish is not considered relevant due to their ability to rapidly metabolise these compounds enzymatically within the liver before being excreted via the bile (Hellou and Payne, 1987; Varanasi et al., 1989; Collier et al*.,* 1996; Hellou and Upshall, 1995). Determination of FACs in fish bile has become a well established method as a rapid and sensitive indicator of exposure of petroleum hydrocarbons (Krahn et al., 1992; Britviæ et al., 1993). In the present study total FACs were present in barramundi from all river systems sampled; however, it was only in the Johnstone River where these levels were significantly higher than the other four rivers. It is well established that both naphthalene and phenanthrene along with their metabolites are derived from petrogenic sources while the high molecular weight PAH-type metabolites such as benzo(*a*)pyrene are derived from pyrogenic sources (Aas et al., 2000; Barra et al., 2001). The results from this study confirm that barramundi are indeed being exposed to petrogenic sources of PAHs (naphthalene and its metabolites) in the Johnstone River, which is consistent with chemical

analysis and the land use patterns for this catchment. Bile metabolites for benzo(*a*)pyrene (B[*a*]P) were not determined for this study; however, this biomarker will be included in all future field studies as an indicator of exposure to pyrogenic sources of PAHs. For the Johnstone River, with a sizable population (~8000) situated on its banks, impact of urban runoff is of concern because this point source generally contains higher levels of pyrogenic PAHs (Kucklick et al., 1997; Ngabe et al., 2000; van Dolah et al., 2005).

Many environmental contaminants, including PAHs (Ching et al., 2001; Jakšić and Batel, 2003), metals (Schroder et al., 1999) and industrial and urban effluents (Gravato and Santos, 2003) can act as genotoxins, acting directly or after being metabolised to reactive intermediates through the action of the cytochrome P450 monooxygenase system (Nebert and Gonzalez, 1987; Buhler and Williams, 1988). These reactive intermediaries may form DNA adducts (Ching et al., 2001), or free radicals and/or reactive oxygen species forming alkali-labile sites on DNA (Downs et al., 2002). As such changes in the integrity of DNA or damage to genetic material has been suggested as a biomarker of genotoxic compounds (Shugart, 2000). Due to its ease, speed and low cost the Fast Micromethod® has been suggested as a suitable tool to quantify this biomarker for the presence of genotoxins (Batel et al., 1999; Bihari et al., 2002; Jaksic and Batel, 2003). Analysis of genetic damage in fish from the five river systems, by Fast Micromethod®, showed significantly higher levels of strand breakage in fish from the Johnstone River than in fish from the four other rivers. There were a number of potential contaminants found in the Johnstone River that may have bought about this increase in DNA strand breakage in fish liver. Benzo(*a*)pyrene, a well know genotoxin, was found in both sediment and water samples from the Johnstone river at levels high enough to induce DNA damage (Everaarts et al*.,* 1998; Ching et al*.,* 2001).

Acetylcholinesterase (AChE) is present in most vertebrates and is responsible for the deactivation of acetylcholine at nerve endings, preventing continuous firing of nerves. AChE activity is inhibited by organophosphorus and carbamate insecticides and as such has been used extensively to monitor for these compounds in the environment (Galgani et al., 1992; Payne et al., 1996; Bocquene and Galgani, 1998). The inhibition of cholinesterase is generally considered to be specific to organophosphorus or carbamate insecticides (Sturm et al., 1999). Significant comparative inhibition of cholinesterase activity in fish suggests exposure to organophosphorus and carbamate insecticides in the Herbert River and Johsntone Rivers. Chemical analysis of both sediments and water failed to detect either of these classes of compound in any river system, though the presence of diuron in sediments from all samples from these sites suggest that agricultural chemicals are making their way into these systems. Chlorpyrifos, an organophosphorus insecticide, is one of the most heavily used pesticides in both of the Herbert and Johnstone catchments (Hamilton and Haydon, 1996). Failure to detect this compound may just be indicative of the fact that it is not persistent in the environment. Spot sampling (weekly) of sediment in waterways of the New South Wales cotton areas failed to detect chlorpyrifos yet integrated sampling was able to detect chlorpyrifos in these same sites (Anon., 2000). The inhibition of ChE shown in this study may in fact be representative of past exposure to chlorpyrifos that was not detected by spot sampling. Another suggestion, is that ChE in fish from the Johnstone and Herbert Rivers was inhibited by compounds other than organophosphorus insecticides and carbamates, including combustion hydrocarbons. Inhibition of acetylcholinase by these compounds has been proposed by Payne et al. (1996).

The response of different biomarkers in *in situ* organisms often fluctuate in response to the state of health of the organism, the presence of different mixtures of contaminants and the different environmental conditions found between sites, often making the data extremely difficult to interpret. Multivariate analysis techniques are an extremely useful tool for the interpretation of biomarker data in that they are able to maximise inter-site variance and produce two-dimensional patterns of similarity between different groups of data (van der Oost et al., 1997; Astley et al., 1999).

The multi-variate analysis conducted for this study helped elucidate the relationships between multiple biomarkers in multiple locations and the chemical contaminants present at each site. If a multi-biomarker approach is to be continued it is important to find suites of markers that complement each other and adequately characterise the ecosystem being studied. The five estuaries in the present study exhibited different pollution patterns and levels, ranging from the 'clean' Lockhart and Pascoe estuaries to the more 'polluted' Johnstone and Herbert Estuaries. The principal component analysis and discriminant analysis performed on the biochemical parameters proved to be the most effective method for classifying the pollution status of the different sites. The principal component analysis showed that the biomarkers that better distinguished the sites were EROD, EROD/P450, DNA damage and to a lesser extent ChE and FACs. Future studies will aim to include B[*a*]P metabolites in the analysis of FACs as this could increase the ability of this biomarker to distinguish between the five rivers studied. The fish biometrics and HSI had little impact on distinguishing between sites indicating that in general contaminants are not impacting upon the general fitness of the fish. It is interesting to note that in the tree diagrams of the discriminate analysis of the fish biomarkers and the organic contaminants in the sediment and water the Johnstone and Herbert Rivers group together while the other three rivers separate out to form their own group. These diagrams reflect the land use pattern in each of the catchments as outlined in Table 1.

5. Conclusion

This study has shown that the complimentary use of several biomarkers in barramundi and multivariate analysis have the potential of being extremely effective in evaluating the state of exposure of these organisms to complex mixtures of chemical contaminants in estuaries of northern Queensland. The suite of biomarkers selected complemented each other and were shown to be sensitive to a range of contaminants present in the different systems. In particular the phase I enzymes in barramundi (EROD, EROD/P450), DNA damage and cholinesterase activity were found to be the most responsive to organic contaminants in the environment. Fish biometrics and the condition index and hepatosomatic index were found to be less sensitive biomarkers, though this is likely to be due to the fact that these indicators are at higher levels of organisation and are yet to be affected. Generally effects are initially manifested at lower levels of organisation (e.g. molecular, cellular) though ultimately such low-level effects can exert significant effects on growth and survival of individual organisms, and eventually flow through the population and community measures after continued or increased exposure to contaminants. Discriminant analysis was well suited to distinguish between the various sites, classifying the pollution status of each and helping to examine the relationships between exposure to contaminants and biochemical response in barramundi.

Future studies will be strengthened by measuring the uptake of contaminants into various tissues of barramundi to gain information on the bioavailability and bioaccumulation of the chemical contaminants present within the estuaries, hopefully providing greater insight into the mechanisms, causes and effects of pollution in tropical estuaries. The suite of biomarkers employed by this study could be modified by reducing the number of phase I enzymes investigated and including biomarkers of metals (metallothionein), endocrine disruptors (vitellogenin) and anti-oxidants (GST, SOD).

This study has shown that the integrated use of biomarkers and environmental monitoring provides a quick, easy and cost effective method of assessing the environmental health of estuaries in northern Queensland. Further study will provide greater information on other potential contaminants as well as temporal and spatial patterns of this contamination.

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