Hepatic coenzyme Q redox balance of fishes as a potential bioindicator of environmental contamination by polycyclic aromatic hydrocarbons

Ghitarina Hasbi^{1,2}, Rocky de Nys^{1,2},
Kathryn Burns³, Steve Whalan¹
and Walter C. Dunlap^{3,*}

¹School of Marine and Tropical Biology, and ²AIMS@JCU Tropical Aquaculture, James Cook University, Townsville, Queensland 4811, Australia

³Australian Institute of Marine Science, PMB No. 3, Townsville MC, Queensland 4810, Australia

*Author for correspondence (wdunlap@aims.gov.au).

In this communication, we introduce a novel biomarker of aquatic contamination based on the xenobiotic-induced response of the hepatic coenzyme Q (CoQ) redox balance of fishes to polycyclic aromatic hydrocarbons (PAHs). The method is demonstrated by comparing changes in the liver CoQ redox balance with that measured using the CYP1A-based, 7-ethoxyresorufin-O-deethylase activity assay, on administration of benzo[a]pyrene (BaP) and β -naphthoflavone (BNF) to Barramundi (*Lates calcarifer*). Both assays showed comparable dose-dependent effects in fish treated with BaP or BNF. Perturbation in the constitutive hepatic CoQ redox balance of fishes may thus provide a simple biomarker of aquatic PAH contamination.

Keywords: pollution biomarker; coenzyme Q; EROD; Barramundi

1. INTRODUCTION

Marine and freshwater ecosystems at the land–water interface are often exposed to pollutants derived from anthropogenic sources. Yet, only a few effective methods have been employed routinely to monitor aquatic contamination for environmental risk assessment [1]. Current methods to determine sub-lethal effects of polycyclic aromatic hydrocarbon (PAH) contamination on aquatic ecosystems are often based on measuring the response of fish hepatic microsomal cytochrome P4501a (CYP1A) mono-oxygenase enzyme activities, by quantitative CYP1A protein determination or by measuring CYP1A mRNA expression levels. The monitoring procedure most commonly employed to detect PAH contamination in aquatic ecosystems is the fish hepatic CYP1A-based,

7-ethoxyresorufin-O-deethylase (EROD) enzyme activity assay [2]. While there is ample evidence of the successful use of the EROD assay as an effective environmental biomarker of PAH contamination, the assay can be affected by biotic variabilities and by inconsistencies inherent in the preservation of enzyme catalytic activities during tissue or homogenate storage [3].

We introduce measurement of the fish hepatic coenzyme Q (CoQ) redox balance as an alternative bioindicator of aquatic PAH contamination, based on the physiological affects of PAHs on a redox endpoint of oxidative metabolism, which does not require the determination of a catalytic enzyme activity. In mitochondria, the reduced form of CoQ (ubiquinol; CoQH₂) is oxidized during respiration (m[O₂]) to ubiquinone (CoQ) to generate ATP. For sustained metabolism, CoQH₂ is replenished by reduction of CoQ by the enzyme NAD(P)H : quinone oxidoreductase (NQOR; a.k.a. DT-diaphorase) (figure 1). Accordingly, there are two distinct metabolic responses possible for metabolic perturbation of the constitutive redox balance of the hepatic CoQ pool of fishes exposed to aquatic pollutants: (i) a direct response to aquatic contaminants by the production of reactive oxygen species (ROS), causing a decline of hepatic CoQH₂; and (ii) an increase in hepatic CoQH₂, caused by certain xenobiotic contaminants, such as PAHs, that induce cellular NQOR activity. In this study, we monitor the latter response to examine the effects of benzo[a]pyrene (BaP) and β -naphthoflavone (BNF) on the hepatic CoQ redox balance of Barramundi as a potential bioindicator of PAH contamination, assessed by direct comparison with results obtained from the EROD assay.

2. MATERIAL AND METHODS

Lates calcarifer (Barramundi) is a common tropical Australasian species that inhabits both fresh and estuarine waters. The EROD assay has been well used in Barramundi to examine the impact of PAH contamination in tropical aquatic biotopes [4]. All experimental treatments used sexually immature male juveniles (150–200 g) purchased from a local hatchery (North Queensland Barramundi Pty. Ltd., Townsville). Fish were acclimated to aquaria conditions for two weeks prior to experimental treatment. In all procedures, individual fish were held in a 70 l aquaria containing de-chlorinated freshwater maintained at 28°C under a 12 L : 12 D photoperiod in a recirculating system with continuous aeration; fish were fed daily to satiation on a commercial food pellet.

Barramundi juveniles ($n = 5$) were administered 0.002, 0.02 and 2 mg kg⁻¹ BaP dissolved in corn oil, and individuals were injected with corn oil alone (carrier blanks) or maintained as nil-injected controls. Similarly, BNF was administered to juveniles ($n = 10$) at levels of 5, 10, 20, 50 and 100 mg kg⁻¹ of BNF in corn oil and maintained with appropriate blanks and controls. Following administration, individuals were maintained in separate aquaria for 48 h and were then anaesthetized with clove oil prior to sacrifice by cervical dislocation. Livers were removed directly, weighed and divided equally with one sample stored at -80°C for determination of the CoQ redox balance. The remaining portions were homogenized immediately by standard procedures [4] for the EROD assay and protein determination (electronic supplementary material, file 1). The hepatic CoQ redox balance was determined chromatographically by the procedure described by Gagliano *et al.* [5] (electronic supplementary material, file 2). CoQ redox values are expressed as the per cent ratio, %CoQH₂ = $100 \times ([\text{CoQH}_2]) / ([\text{CoQ}] + [\text{CoQH}_2])^{-1}$. One-way analysis of variance (ANOVA) was used to examine the effect of PAHs on the EROD and CoQ redox assays with assumptions of data normality (Q-Q plot) and homoscedasticity (Levene's test) validated prior to execution. Bonferroni's post hoc test was used to determine significant difference using IBM SPSS Statistics 17.0 software.

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2010.0600> or via <http://rsbl.royalsocietypublishing.org>.

Figure 1. Scheme showing the biochemical components of CoQ metabolism that determine the metabolic poise of the CoQ redox balance. m[O₂], oxidative metabolism; ROS, reactive oxygen species.

3. RESULTS

There was a significant effect of BaP administration (table 1) on Barramundi liver EROD values (figure 2a) on fish injected with 0.002 mg kg⁻¹ BaP. The lowest administered dose of 0.002 mg kg⁻¹ showed a mean EROD activity level ($\pm$ s.e.) of 64.83 ± 5.76 pmol min⁻¹ mg protein⁻¹, which was significantly greater than the EROD activity of control fish without treatment (31.05 ± 1.99 pmol min⁻¹ mg protein⁻¹) and those administered the corn oil blank (27.64 ± 5.01 pmol min⁻¹ mg protein⁻¹). The higher BaP dosages of 0.02 and 2 mg kg⁻¹ gave diminished EROD values (51.42 ± 7.65 and 45.85 ± 6.50 pmol min⁻¹ mg protein⁻¹, respectively) that were not significantly different from EROD activities of the nil-injected control. There was no significant difference in the hepatic EROD activities of the nil-injected control fish and those injected with the corn oil blank, showing that there was no effect of the injection carrier in the experimental procedure.

Barramundi juveniles exposed to BaP responded also by elevation of hepatic %CoQH₂ redox values (figure 2b; table 1). The greatest hepatic CoQH₂/total CoQ ratios (%CoQH₂ $\pm$ s.e.) were present in fish injected with 0.002 mg kg⁻¹ BaP (%CoQH₂ = 88.59 ± 2.40) and 0.02 mg kg⁻¹ BaP (%CoQH₂ = 88.09 ± 2.08). Similar to the EROD results, both doses gave %CoQH₂ values that were significantly greater than that of control fish (%CoQH₂ = 78.61 ± 1.05) and those administered the corn oil blank (%CoQH₂ = 77.14 ± 1.58). Fish treated with the greatest experimental dose of BaP (2 mg kg⁻¹) gave a diminished %CoQH₂ value (%CoQH₂ = 85.71 ± 2.11) that, as in the EROD assay, was not significantly different from the 0.002 and 0.02 mg kg⁻¹ BaP dosages or controls. There were no significant differences in the hepatic %CoQH₂ redox values of nil-injected controls and those injected with the corn oil blank.

There was likewise a significant effect of BNF administration (table 1) on Barramundi liver EROD activities (figure 2c). The mean hepatic EROD value ($\pm$ s.e.) for fish injected with the lowest dose of 5 mg kg⁻¹ showed a hepatic EROD activity (15.45 ± 1.67 pmol min⁻¹ mg protein⁻¹) that was not significantly greater than that of control fish without BNF administration (10.71 ± 1.17 pmol min⁻¹ mg protein⁻¹). BNF doses of 10, 20 and 50 mg kg⁻¹ gave dose-dependent increases in EROD activities (18.22 ± 1.87 , 27.21 ± 1.08 and 39.21 ± 0.99 pmol min⁻¹, respectively) that were

Table 1. Summary of ANOVA results showing effects of BaP and BNF on Barramundi liver EROD and %CoH₂ values. (d.f., degrees of freedom.)

assay/ PAH	sum of squares	d.f.	mean square	F	p
EROD					
BaP					
dose	4643.96	4	1160.99	7.03	0.001
error	3303.47	20	165.17		
BNF					
dose	5762.30	5	1152.46	74.18	<0.001
error	838.91	54	15.54		
%CoH ₂					
BaP					
dose	737.49	4	184.37	10.23	<0.001
error	360.61	20	18.03		
BNF					
dose	2377.48	5	475.50	33.05	<0.001
error	776.81	54	14.39		

significantly greater than that of control fish without BNF treatment. The EROD activity of fish injected with the maximum dose of 100 mg kg⁻¹, however, gave an EROD activity (31.22 ± 1.27 pmol min⁻¹ mg protein⁻¹) that was significantly less than the maximum EROD activity measured at a BNF dose of 50 mg kg⁻¹, and was similar to the response measured at the lower BNF dose of 20 mg kg⁻¹.

Barramundi responded also to BNF treatment by elevation of hepatic %CoQH₂ redox values (figure 2d; table 1). As in the EROD assay, the hepatic redox balance of fish treated with the lowest dose of 5 mg kg⁻¹ BNF (%CoQH₂ = 74.28 ± 1.61) was not significantly greater than that of control fish without BNF treatment (%CoQH₂ = 72.67 ± 1.82), whereas fish administered BNF at doses of 10, 20, 50 and 100 mg kg⁻¹ showed a significant increase in hepatic %CoQH₂ redox values (%CoQH₂ = 83.45 ± 1.14 , 88.07 ± 1.32 , 88.28 ± 1.48 and 85.60 ± 1.80 , respectively) that were not statistically different across these higher dosages.

4. DISCUSSION

There are few published studies to have examined the metabolic biochemistry of CoQ in fishes [6,7],

Figure 2. The effects of BaP on *L. calcarifer* ($n = 5$) 48 h post-administration; (a) quantified as the mean EROD activity ($\pm$ s.e.); (b) quantified as the mean %CoQH₂ redox value ($\pm$ s.e.), CO is the corn oil blank without BaP. The effect of BNF on *L. calcarifer* ($n = 10$) 48 h post-administration; (c) quantified as the mean EROD activity ($\pm$ s.e.); (d) quantified as the mean %CoQH₂ redox value ($\pm$ s.e.).

Recently, Gagliano *et al.* [5] compared the CoQ isoform (isoprenoid length) composition and the hepatic CoQ redox status of native specimens across families of coral reef fishes. This study found little variation in the constitutive CoQ redox status of native conspecifics, although differences occurred among fishes of diverse genera, demonstrating that the homeostatic liver CoQ redox balance within a natural conspecific population offers a stable baseline from which xenobiotic-mediated perturbations can be measured.

Administration of BaP and BNF gave a significant increase in the hepatic %CoQH₂ redox response of Barramundi (figure 2b,d) consistent with BaP and BNF activation of hepatic NAD(P)H : quinone oxidoreductase reported in the rainbow trout, *Oncorhynchus mykiss* [8], presumably to protect against contaminant-induced oxidative damage [9] by enhancing the rate of ubiquinone reduction to provide greater concentrations of the cytoprotective ubiquinol antioxidant [10]. Furthermore, the remarkable relationship observed between hepatic CoQ redox balance and EROD enzyme activity of fishes is linked possibly through an oxidative stress-mediated pathway [11,12] and, although not measured in this study, the induction of hepatic NQOR activity is implicit to the observed increases of liver %CoQH₂ in Barramundi juveniles administered BaP or BNF.

Comparison of the hepatic %CoQH₂ redox balance and the EROD response of Barramundi provides an empirical basis for applying the fish liver CoQ redox determinant as a potential biomarker of aquatic PAH contamination. Practical use of the fish liver CoQ redox balance as a bioindicator of aquatic pollution, however, has yet to be validated as a suitable response to PAH exposure in field determinations. Examination is also needed to establish potential sources of biotic and abiotic interferences, such as pro-oxidants that do not activate NQOR induction and toxicants that may repress NQOR activity by redox cycling. For example, fishes prone to high rates of respiration during intense pelagic activity can evoke acute tissue hypoxia in myotomal muscle, which may alter liver metabolism affected by high influxes of plasma lactic acid concentrations [13]. The hepatic CoQ redox balance is, nevertheless, a robust analytical measure since the determination is based on a concentration ratio, rather than an absolute enzyme activity determination, since extraction inconsistencies are normalized by near-identical extraction efficiencies of the CoQ and CoQH₂ redox components [14].

The utility of the hepatic CoQ redox response of fishes as a biomarker of environmental PAH contamination on administration of benzo[a]pyrene and BNF to juvenile Barramundi is demonstrated empirically

by direct comparison with the EROD assay. We contend that the determinant of the hepatic CoQ redox balance of fishes has potential, based on firm physiological principles, to be used as a practical biomarker of PAH contamination in aquatic biotopes.

Our research was conducted in compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and the Queensland Animal Care and Protection Act 2001 under appropriate ethics guidelines prescribed by the James Cook University Animal Ethics Committee, approval no. A855 (2003–2005) and A1121 (2006).

This project was funded by the Australian Research Council (DP0450425) with support of the AIMS@JCU programme of the James Cook University and the Australian Institute of Marine Science. G.H. was supported by an AusAID scholarship.

- 1 Van der Oost, R., Beyer, J. & Vermeulen, N. P. E. 2003 Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol.* **13**, 57–149.
- 2 Whyte, J., Jung, R., Schmitt, C. & Tillit, D. 2000 Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit. Rev. Toxicol.* **30**, 347–570. (doi:10.1080/10408440091159239)
- 3 Krüner, G. & Westernhagen, W. V. 1999 Sources of measurement error in assays of EROD activity of fish for biological effects monitoring. *Helgol. Mar. Res.* **53**, 250–256.
- 4 Humphrey, C. C., Codi-King, S. & Klumpp, D. W. 2007 A multibiomarker approach in barramundi (*Lates calcarifer*) to measure exposure to contaminants in estuaries of tropical North Queensland. *Mar. Pollut. Bull.* **54**, 1569–1581. (doi:10.1016/j.marpolbul.2007.06.004)
- 5 Gagliano, M., Dunlap, W. C., de Nys, R. & Depczynski, M. 2009 Ockham's razor gone blunt: coenzyme Q adaptation and redox balance in tropical reef fishes. *Biol. Lett.* **5**, 360–363. (doi:10.1098/rsbl.2009.0004)
- 6 Giardina, B., Gozzo, M. L., Zappacosta, B., Colacicco, L., Callà, C., Mordante, A. & Lippa, S. 1997 Coenzyme Q homologs and trace metal element content of Antarctic fishes *Chionodraco hamatus* and *Pugotheria bernicchi* compared with the Mediterranean fish *Mugil cephalus*. *Comp. Biochem. Physiol. A* **118**, 977–980. (doi:10.1016/S0300-9629(97)86785-0)
- 7 Souchet, N. & Laplante, S. 2007 Seasonal variation of co-enzyme Q10 content of pelagic fish tissues from Eastern Quebec. *J. Food Comp. Anal.* **20**, 403–410. (doi:10.1016/j.jfca.2006.09.004)
- 8 Sturve, J., Stephensen, E. & Förlin, L. 2005 Effects of redox cycling compounds on DT diaphorase activity in the liver of the rainbow trout (*Oncorhynchus mykiss*). *Comp. Hepatol.* **4**, 4. (doi:10.1186/1476-5926-4-4)
- 9 Livingstone, D. R. 2001 Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar. Pollut. Bull.* **42**, 656–666. (doi:10.1016/S0025-326X(01)00060-1)
- 10 Dinka-Kostova, A. T. & Tatalay, P. 2000 Persuasive evidence that quinone reductase type 1 (DT-diaphorase) protects cells against the toxicity of electrophiles and reactive forms of oxygen. *Free Radic. Biol. Med.* **29**, 231–240.
- 11 Marchand, A., Barouki, R. & Garlatti, M. 2004 Regulation of NAD(P)H: quinoine oxidoreductase 1 gene expression by CYP1A1 activity. *Mol. Pharmacol.* **65**, 1029–1037. (doi:10.1124/mol.65.4.1029)
- 12 Timme-Laragy, A. R., Van Tiem, L. A., Linney, E. A. & Di Giulio, R. T. 2009 Antioxidant response and NRF2 in synergistic development toxicity of PAHs in zebrafish. *Toxicol. Sci.* **109**, 217–227. (doi:10.1093/toxsci/kfp038)
- 13 Wells, R. M. G. & Baldwin, J. 2006 Plasma lactate and glucose flushes following burst swimming in silver trevally (*Pseudocaranx dentex*: Carangidae) support the 'releaser' hypothesis. *Comp. Biochem. Physiol. A* **143**, 347–352.
- 14 Yamamoto, Y. & Yamashita, S. 1997 Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress. *Anal. Biochem.* **250**, 66–73.