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Biliary PAH Metabolite Elimination in Australian Bass, *Macquaria novemaculeata* Following Exposure to Bass Strait Crude Oil and Chemically Dispersed Crude Oil

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The remediation technique used to clean up an oil spill plays a major role in determining its potential ecological impact. Chemically dispersing crude oil is one of the more commonly used options available. Following application of dispersants to an oil slick, the total petroleum hydrocarbon (TPH) concentrations in the water column can reach up to 10 ppm (Lunel et al., 1995), potentially causing acute toxic effects on biota (Cohen and Nugegoda, 2000). An alternative option is to allow the floating oil to naturally weather on the sea surface. This technique has been shown to have less detrimental effects on invertebrates and fish, than chemically dispersing the oil, since TPH concentrations in crude oil water accommodated fraction (WAF) were 4-5 lower than in the dispersed oil WAF (Fucik, 1994).

Effects from exposure to petroleum hydrocarbons include fish tainting (Zhou et al., 1997), altered growth (Connolly and Jones, 1996) and deformities in larval fish (Collier et al., 1996). A previously applied method for examining hydrocarbon exposure to fish was the analysis of petroleum hydrocarbons in tissue (Short et al., 1996). However, because fish readily metabolise and depurate hydrocarbons, it has been difficult to relate body burden to petroleum hydrocarbon exposure concentrations (Collier et al., 1996).

Biotransformation of xenobiotics such as petroleum hydrocarbons (PAHs) is catalysed by mixed function oxidase (MFO) enzymes, followed by conjugation of the free metabolites by Phase II enzymes. The water soluble metabolites are directed to the bile where they are concentrated and eliminated (Varanasi et al., 1989). PAH metabolites in bile, because of their fluorescent properties have proven useful in assessing the exposure of fish to petroleum hydrocarbons (Krahn et al., 1992).

The present study investigated benzo(a)pyrene-type (B(a)P-type) and naphthalene-type metabolite elimination in Australian bass, *Macquaria novemaculeata*, following waterborne exposure to Bass Strait crude oil and chemically dispersed crude oil. Australian bass is a commercially and recreationally important species, which has proven to be an excellent indicator for monitoring the effects of exposure to petroleum hydrocarbons (Cohen and

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Nugegoda, 2000). The aim of the experiment was to expose the test species to the water accommodated fraction (WAF) of Bass Strait crude oil and dispersed crude oil and evaluate biliary B(a)P-type and naphthalene-type metabolite elimination following exposure and during depuration in clean seawater.

MATERIALS AND METHODS

Juvenile Australian Bass were obtained from Searle Aquaculture, N.S.W, and transported to the Queenscliff Marine Station, Victoria in oxygenated bags. Prior to experimentation organisms were transferred randomly across six 80-litre tanks with closed lids and acclimatised for 2 weeks.

Three treatments were set up (crude oil water WAF, dispersed crude oil WAF, and control), with two replicates per treatment. Only two replicates per treatment were chosen based on logistical constraints. The method of obtaining the WAF was adopted from Anderson et al. (1974). Crude oil and seawater (1:9) were mixed for 20 hours and the solution left to settle for 1 hour before isolating the WAF. Crude oil WAF and dispersed crude oil WAF were delivered to the experimental tanks using a peristaltic pump which resulted in a treatment concentration that was 2% of the original WAF stock solutions.

Fish were exposed to seawater (control), to the WAF of Bass Strait crude oil and chemically dispersed oil for 4 days followed by a 12 day depuration period. Organisms were stocked at 15 organisms/tank, resulting in a loading rate of 1.2 g/ L of water. Five fish were sampled from each tank on days 0, 4 and 16. TPH concentrations were analysed by taking 250ml samples from the 100% WAF preparations and extracting twice with 25ml lots of analytical reagent grade dichloromethane. Sodium sulphate was added to the extracts for dehydration. Samples were stored at -20^0C and then analysed using gas chromatography (GC-FID).

The gall bladders of individual *M. novemaculeata* were collected and stored in liquid nitrogen. Fixed fluorescence (FF) detection (Lin et al., 1996) was used for quantifying biliary metabolites. Bile samples were firstly diluted to 1:100 (bile:distilled water) for protein analyses (Lowry et al., 1951). This dilution was used to obtain adequate sample volume. A 1:100 dilution was used for B(a)P-type metabolite measurements and samples further diluted to 1:1000 for naphthalene-type metabolite measurements.

Samples were analysed using a spectrofluorimeter at the set wavelength of 380/430nm for B(a)P-type and 290/335nm for naphthalene-type metabolites. A slit width set at 5nm was used for both excitation and emission. Naphthalene-type and B(a)P-type metabolites were quantified using 1-naphthol and 1-pyrenol as fluorescent standards. Biliary metabolites are reported as μg of a particular metabolite per mg protein in the bile (Lin et al., 1996). Naphthalene-type and B(a)P-type metabolites were used as these biliary metabolites are established biomarkers for monitoring PAH elimination in fish (Lin et al., 1996).

A means versus standard deviations plot was used to examine homogeneity of variances (Statistica, Version 5). B(a)P-type metabolite data and naphthalene-type metabolite data were log transformed to achieve normality. Two-way nested ANOVA's were then performed, with treatment and day as independent variables and replicate nested within treatment. A Tukey HSD test was then performed if there was significant differences between treatments, to identify differences between treatments.

RESULTS AND DISCUSSION

Physicochemical parameters measured daily in the exposure chambers (mean ± S.D., n=16) were: temperature 18±0.6 ^{0}C; salinity 35±0.2 ppt; dissolved oxygen 6.4±0.2 mg/l; pH 8.3±0.3. The measured TPH concentrations in the 100% oil WAF and dispersed oil WAF stock solutions were 3.4 mg/l and 19.2 mg/l. Therefore nominal concentrations in the dispersed crude oil WAF and crude oil WAF experimental tanks were 384 μg/l and 68 μg/l respectively.

TPH concentration was analysed in the 100% preparations rather than the experimental tanks, because nominal concentrations in the tanks were estimated as low as, which is considered too low for accurate measurement using GC. However, the use of peristaltic pumps during experimentation ensured hydrocarbon concentrations in the exposure chambers remained constant 24 hours per day. Previous research indicates that even in static conditions where gentle aeration was used, no significant loss of hydrocarbons in experimental tanks occurred over the exposure period (Cohen and Nugegoda, 2000).

Chemically dispersing crude oil resulted in five times higher concentrations of TPH in the water column, compared to undispersed crude oil. This has also been shown by Fucik (1994), where the TPH concentration analysed in the dispersed oil WAF stock solution was several times higher than the concentration analysed in the non-dispersed crude oil. When dispersant is applied to an oil slick, the oil is broken up into oil droplets dispersing into the water column (Gilbert, 1996).

Statistical analysis of the data using ANOVA revealed a significant effect from the interaction between day and treatment on concentrations of naphthalene-type ($p=0.012$) and B(a)P-type ($p=0.001$) biliary metabolites. Therefore, results are presented per day, per treatment. Both types of biliary metabolites in fish from the crude oil WAF and dispersed oil WAF treatments were significantly higher than in control fish after 4 days exposure (Fig.1).

There was no significant difference in PAH biliary metabolite concentrations after four days, between the crude oil WAF and dispersed oil WAF treatments ($p=0.579$ for B(a)P-type and $p=0.999$ for naphthalene-type biliary metabolites). After only four days of exposure, B(a)P-type metabolite concentrations in the crude oil WAF and dispersed crude oil WAF were 1.47 ± 0.32 μg/mg protein and 1.74 ± 0.39 μg/mg protein respectively (Mean ± S.E.M.). After 12 days depuration in clean seawater, B(a)P-type metabolites remained higher in fish

previously exposed to crude oil WAF, relative to fish from the dispersed oil WAF and controls (p=0.018).

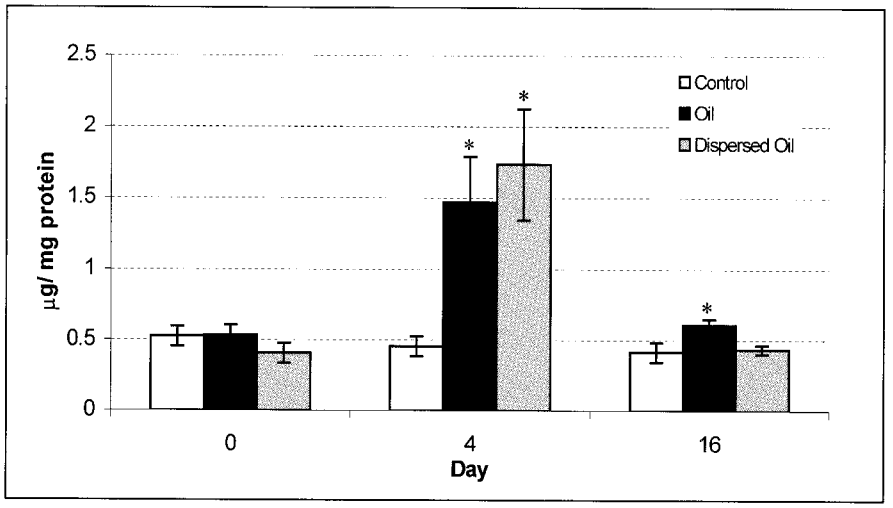

Figure 1. B(a)P-type metabolite concentrations (µg/mg protein) measured in the bile of Australian Bass, exposed for 4 days to Bass Strait crude oil WAF and dispersed crude oil WAF, followed by 12 days depuration in clean seawater (Mean ± S.E.M., n=10 fish/ treatment/day). * Indicates significant difference of the mean relative to the mean of control fish on that day at $p<0.05$.

Relative to control fish, fish in the crude oil WAF and dispersed crude oil WAF treatments had significantly higher naphthalene-type metabolite concentrations after the 4 day exposure to the petroleum compounds (p=0.005) (Fig. 2).

On day 4 of the experiment, naphthalene-type metabolite concentrations in the crude oil WAF and the dispersed oil WAF treated fish were 1936 ± 254 µg/mg protein and 2442 ± 382 µg/mg protein, respectively (Mean ± S.E.M.). However, after the 12 days of depuration in clean seawater, naphthalene-type metabolites in fish treated with crude oil WAF and dispersed crude oil WAF returned to control levels (p=0.118).

Results of the present study showed that exposure of fish to petroleum hydrocarbons can be detected as early as 4-days following the onset of exposure. Previous research has found bile metabolites reach a maximum in fish exposed to No. 2 Fuel Oil, 6 days after initial exposure under laboratory conditions (Hellou and Payne, 1987). In the present study, fish treated for 4 days with crude oil WAF had similar biliary PAH metabolite concentrations to fish in the dispersed oil WAF treatment. It is assumed that elimination of biliary metabolites is proportional to uptake and therefore this similarity between treatments may be attributed to the detoxification system reaching a maximum rate following short-term exposure (Hellou and Payne, 1987).

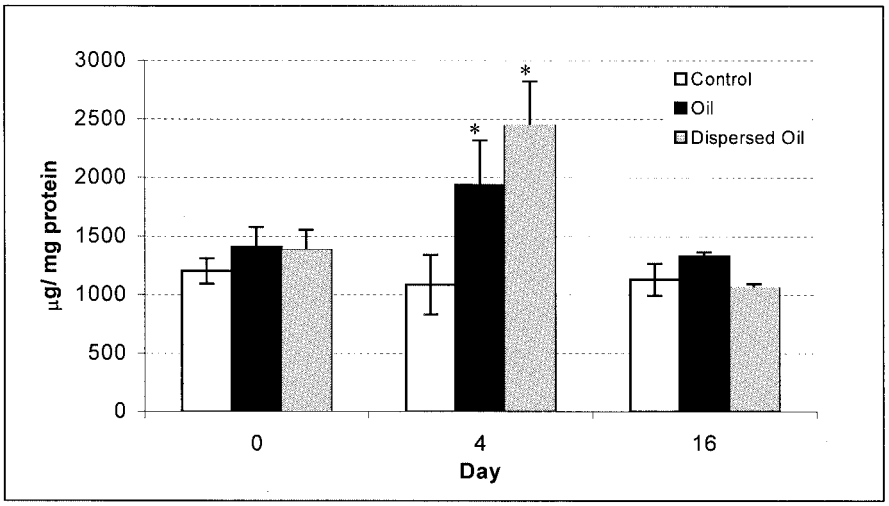

Figure 2. Naphthalene-type metabolite concentrations (µg/mg protein) measured in the bile of Australian Bass, exposed for 4 days to Bass Strait crude oil WAF and dispersed crude oil WAF, followed by 12 days depuration in clean seawater (Mean ± S.E.M., n=10 fish/ treatment). * Indicates significant difference of the mean relative to the mean of control fish on that day at $p<0.05$.

Previous research has investigated the persistence of PAH metabolites in the bile of benthic fish species, during low but chronic exposure to crude oil. Collier et al. (1996) found phenanthrene metabolites persisting in yellowfin sole, *Pleuronectes asper*, rock sole (*Pleuronectes bilineatus*) and flathead sole (*Hippoglossoides elassodon*) for three years after the *Exxon Valdez* oil spill. It is expected that tidal flushing is greatest in the mid to upper water column and therefore subsistence of hydrocarbons would be minimal in this environment. Collier et al. (1996) found that in the only pelagic species investigated in their study, Dolly varden (*Salvelinus malma*), there was limited persistence of PAH metabolites one year after the oil spill.

The present study demonstrated that after 12 days depuration in clean seawater, naphthalene-type metabolite concentrations in the bile of Australian Bass (a pelagic species) returned to background levels, which indicates limited persistence of this metabolite in a clean environment. B(a)P-type metabolites were more persistent however, since they were still higher in fish exposed to crude oil WAF after the 12-day depuration period relative to unexposed fish. This enhanced persistence of B(a)P-type metabolites, may be attributed to the higher molecular weight of the latter compound, in comparison to naphthalene. The persistence of B(a)P-type metabolites in the crude oil WAF treatment may also be due to significantly lower metabolic activity resulting in persistence of B(a)P-type metabolites, compared to the dispersed crude oil WAF treatment, which showed enhanced metabolic activity and a higher degree of sub-lethal stress (Cohen et al., 2001).

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