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Effect of crude oil contaminated sediment exposure on cytochrome P450 enzymes in the Australian asteroid *Coscinasterias muricata*

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Abstract

Levels of cytochrome P450 enzymes were measured in pyloric caeca microsomes of the asteroid *Coscinasterias muricata* following exposure to sediment with nominal concentrations of 0, 0.1 or 2 ml crude oil kg^{-1} (dry weight) and subsequent depuration. No significant differences were observed in total cytochrome P450 levels or cytochrome P418 levels following the exposure period. However after five days of depuration, levels of total P450 in the pyloric caeca of *C. muricata* exposed to the highest oiled sediment concentration were significantly lower than in specimens exposed to the other treatments. Cytochrome P418 levels were inversely related to total P450 levels following exposure and subsequent depuration. Preliminary results show that levels of CYP1A-like immunopositive protein (CYP1A-like IPP) in exposed asteroids exhibited a concentration response relationship following the exposure period. Variations in CYP1A-like IPP levels observed during the depuration period may be influenced by the sublethal toxicity of hydrocarbons within the crude oil.

Keywords: Bioindicator; CYP1A; Crude oil; Echinodermata

1. Introduction

If the aims of a specific biomonitoring program were to determine temporal or geographic trends of a contamination event, then some echinoderm species would serve as ideal environmental sentinel organisms. By moderating competition among their prey species, top predatory asteroids, such as *Coscinasterias muricata*, maintain a high biodiversity in their benthic communities (Palumbi and Freed, 1988; Edgar, 1997). Interaction suppression or removal of interactive species by the introduction of contaminants into the field may result in indirect community effects leading to deleterious changes to community structure (Bond, 1994). In addition, *C. muricata* has the following traits that make it amenable for use as an environmental sentinel organism; a wide geographical distribution (found in a variety of habitats from reef to soft sediment), a restricted territory, easy collection, large amounts of tissue for analyses, and well known biochemical and physiological processes.

Though biochemical/physiological markers represent a short-term response of relatively low ecological relevance,

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their alteration due to pollution impact, such as modulation of enzyme levels, may be used as early warning systems for the identification of pollutant exposure (Payne et al., 1987). The presence of biotransformation enzymes that convert lipophilic xenobiotics to more water-soluble metabolites is ubiquitous throughout the animal kingdom (Livingstone, 1993). A critical step in the determination of biological activity of numerous xenobiotics, through either activation or inactivation, is the oxidative metabolism by cytochrome P450 biotransformation enzymes (Stegeman et al., 1987). Induction of certain isoforms of this enzyme superfamily (e.g. CYP1A) may indicate exposure to biochemically significant levels of specific xenobiotics, and suggests an enhanced potential for the bioactivation of procarcinogens (Stegeman et al., 1987). The cytochrome P450 isoform CYP1A plays an integral role in the metabolism of organic xenobiotics such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins (Livingstone, 1993).

Induction of total cytochrome P450 and the isoform CYP1A following PAH exposure in both laboratory and field studies has been well documented in marine and freshwater fishes (Buhler and Wang-Buhler, 1998; Whyte et al., 2000). Measurements of total cytochrome P450 and CYP1A activity (measured as benzo[*a*]pyrene hydroxylase (BPH) and the CYP1A-like immunopositive protein (CYP1A-like IPP)), have been studied in a number of laboratory and field studies examining the utility of the asteroid *Asterias rubens* as a bioindicator species of PAH and PCB contamination in the Northern Hemisphere (den Besten, 1998; den Besten et al., 2001; Stronkhorst et al., 2003; Danis et al., 2004). In the Southern Hemisphere, the influence of the reproductive cycle of *C. muricata* on cytochrome P450 levels has been shown to be similar to that observed in fish, whereby decreases in the total level were observed in female specimens prior to and during spawning periods (Georgiades et al., 2005).

Recently, den Besten et al. (2001) and Stronkhorst et al. (2003) found elevated levels of cytochrome P418 (a secondary peak evident between 420 and 416 nm in total cytochrome P450 spectra) in asteroids sampled near pollution sources from the North Sea. Cytochrome P418 is a haemoprotein of unknown function found in marine invertebrates such as echinoderms, molluscs and crustaceans (den Besten et al., 2001). In mammals, the peak at 420 nm is said to be a product of denatured P450 (Livingstone et al., 1989), however, the seasonal occurrence of the secondary peak in rodents and freshwater fish by Rocha-e-Silva et al. (2001) led these authors to suggest that this peak represented a functional protein rather than a degradation product.

The study of the impact of hydrocarbon contamination on aquatic species of the Southern Hemisphere is important as species sensitivities and changes to community structure may differ from those examined in the Northern Hemisphere. The potential for *C. muricata* to serve as a bioindicator species of hydrocarbon contamination has previously been investigated, though these studies primarily focused on asteroid behaviour following water-borne hydrocarbon exposure (Temara et al., 1999; Georgiades et al., 2003). Hydrocarbons, such as PAHs, in marine sediments require attention due to their cytotoxic and potentially carcinogenic properties (Payne et al., 1988). To date no laboratory studies examining the impact of hydrocarbon contaminated sediment on asteroid detoxification enzymes exist.

The current study provides preliminary data on the suitability of total cytochrome P450, the P418 peak, and CYP1A-like IPP as biomarkers of PAH contamination in the asteroid *C. muricata*. The levels of these potential biomarkers were monitored following laboratory exposures to nominal concentrations of oiled sediment. The findings may aid in the establishment of this asteroid as a sentinel species for biomonitoring hydrocarbon contamination in South Pacific waters.

2. Materials and methods

2.1. Collection of asteroids

C. muricata (wet weight 468 ± 100 g, long-radius: the distance from the longest arm tip to the opposite inter-radius 18 ± 1.6 cm, mean ± SD) were collected from Portarlington Pier, Port Phillip Bay, Australia and transported to the Queenscliff Marine Station. Specimens were acclimated to laboratory conditions in 1000 l flow-through aquaria for 18 d (12.3 ± 0.4 °C, salinity 35.6 ± 0.1 ppt, DO >85%) and fed mussels *Mytilus edulis* (L.) *ad libitum*. The collection and exposure period of these asteroids occurred during July/August 2001 (Austral winter) to avoid gender differences in total cytochrome P450 measurements observed in this species during the Austral summer spawning period (Georgiades et al., 2005, in press). Portarlington is within the daily exchange zone of Port Phillip Bay and thus considered a relatively clean sampling site.

2.2. Oiled sediment exposure

C. muricata were exposed to oiled sediment via direct contact with the substrate. Asteroids were exposed in duplicated aquaria ($n = 10$ per aquarium) to nominal concentrations of 0 (control), 0.1 (low concentration) or 2 ml (high concentration) of Bass Strait stabilised crude oil per kg of sediment in aerated, flow-through (2 l min^{-1}) aquaria (120 × 120 × 15 cm). To ensure that asteroids were in constant contact with the substrate, the exposure aquaria were purposely manufactured to be shallow. After five days of exposure, five asteroids from each tank were sampled and dissected ($n = 10$ per concentration). Pyloric caeca required for biochemical analyses were placed into cryovials and immediately frozen in liquid nitrogen to be stored at −80 °C. Asteroid gender was determined by histological analysis of the gonads (Georgiades et al., in press). The remaining asteroids from exposure tanks were pooled according to exposure concentration and placed in separate 40 l flow-through aquaria for depuration to allow for the