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Markers of oil exposure in cold-water benthic environments: Insights and challenges from a study with echinoderms

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ABSTRACT

In spite of increasing naval activities and petroleum exploration in cold environments, there is currently a paucity of tools available to monitor oil contamination in boreal marine life, especially in sedentary (non-fish) species that dominate benthic communities. This research aimed to identify biotic sources of variation in biomarkers using subarctic echinoderms, and to identify suitable biomarkers of their exposure to hydrocarbons. The focal species included the sea star *Asterias rubens*, the brittle star *Ophiopholis aculeata*, the sea urchin *Strongylocentrotus droebachiensis*, and the sea cucumber *Cucumaria frondosa*, which are among the most abundant echinoderms in the North Atlantic and Arctic Oceans. The latter two species are also commercially exploited. A series of 96-h acute exposures of the water-accommodating fraction (WAF) of used lubricating oil (ULO) were performed in different seasons (i.e. distinct reproductive stages). Digestive and reproductive tissues were analyzed for baseline and response levels of glutathione peroxidase (GPx) and ethoxyresorufin-*O*-deethylase (EROD). GPx activity was detected in the pyloric caecum, stomach, and gonad of sea stars, the intestine and gonad of sea cucumbers, and the gonad of brittle stars and sea urchins. No seasonal variation in baseline GPx activity occurred. Upon exposure to the ULO WAF, sex-based differences were elicited in the GPx activity of sea star stomachs (lower in females than males). EROD activity was present in the pyloric caeca of sea stars, and the gonads of brittle stars and sea urchins. An interaction between season and sex on baseline EROD activity was measured in the gonads of sea urchins. Ovaries exhibited significant seasonal variation in EROD activity and had greater activity than testes during the spawning and post-spawning seasons. Seasonal variation in EROD activity also occurred in sea star pyloric caeca and brittle star gonads. Furthermore, testes of sea urchins exposed to the ULO WAF exhibited suppressed EROD activity compared to baseline levels. The nearly universal presence of GPx activity highlights its potential as a useful biomarker, while EROD activity was much more limited. Findings suggest a complex relationship between temporal and biotic factors on both the baseline and response levels of enzymatic activity, emphasizing the need to consider sex and sampling season in studies of biomarkers of hydrocarbon exposure in boreal indicator species that display annual reproductive cycles.

1. Introduction

Diagnostic tools developed for assessing the effects of xenobiotics, including hydrocarbons, in the marine environment tend to be taxonomically and latitudinally biased. For instance, while there has been an increased interest in researching species from polar environments, they still remain understudied relative to species originating from tropical and warm temperate regions (Hansen et al., 2013; Martinez-Gomez et al., 2010; Payne et al., 2003; Sandrini-Neto et al., 2016; Sundt et al., 2012; Thain et al., 2008). Furthermore, tools for assessing the effects of hydrocarbons on macro-invertebrates other than mollusks and crustaceans are limited, despite their predominance in marine ecosystems, and cold-water species are particularly underrepresented (Bechmann et al., 2010; Hannam et al., 2010; Regoli et al., 2002; Sandrini-Neto et al., 2016). This occurs in spite of a growing need for reliable biomarkers of human activity and climate change in arctic and subarctic environments (Nahrgang et al., 2013; Sandrini-Neto et al., 2016). Polar and temperate-cold regions are exposed to marked environmental variations (Sheehan and Power, 1999), making them especially prone to seasonal changes in biological activities (i.e. feeding, growth, and reproduction), which have been shown to influence the expression of biomarkers (Shaw et al., 2004). Hence, there is a particularly strong need to study changes in baseline biomarker activity and response to anthropogenic effects separately across multiple

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seasons in boreal species (Nahrgang et al., 2013).

While mollusks and crustaceans are the most common invertebrate taxa studied and have shown promising results (Baussant et al., 2009; Fossi et al., 2000; Morales-Caselles et al., 2008a, 2008b; Nahrgang et al., 2013), their ability to rapidly metabolize some xenobiotics may not be ideal in terms of bioindicator species (Koenig et al., 2012). Where biochemical studies have been carried out on other tropical and temperate marine invertebrates (i.e. annelids, crustaceans, echinoderms), direct correlation of responses to hydrocarbons have sometimes been weak or absent (den Besten, 1998; Fossi et al., 2000; Payne and May, 1979; Pérez et al., 2004; Snyder, 2000; Solé and Livingstone, 2005). At present, information is scarce on the optimal conditions for sample collection/processing, due to variations in biotic and abiotic factors (Shaw et al., 2004; Sheehan and Power, 1999; Viarengo et al., 1991), and the identification of suitable biomarkers in cold-water species necessitates a case-by-case approach (Morales-Caselles et al., 2008a).

Biomarkers, including glutathione peroxidase (GPx) and ethoxyresorufin-*O*-deethylase (EROD), are commonly used to detect the first signs of biological exposure to adverse compounds (Nahrgang et al., 2013). These assays focus on different enzymatic processes, covering a broad scope of putative responses, and standard techniques are already available to measure them routinely. GPx is a mitochondrial antioxidant enzyme (Morales-Caselles et al., 2008b) that removes hydrogen peroxide (H_2O_2) and organic hydroperoxides which may cause oxidative damage (Doyotte, 1997). Significant dose-dependent increase in activity of GPx in the visceral mass of the gastropod mollusk *Austrocochlea porcata* has been observed after exposure to crude oil (Reid and MacFarlane, 2003). Additionally, Gamble et al. (1995) found GPx in the pyloric caeca of the sea star *A. rubens*. Since, the prevention of oxidative damage is beneficial to all types of tissues, it is expected that GPx activity will be widespread within most marine organisms. On the other hand, EROD is commonly used to measure cytochrome P450 1A (CYP450 1A) activity (Sarkar et al., 2006). In fish from polluted estuaries, levels of hepatic EROD were increased by three classes of compounds: polycyclic aromatic hydrocarbons (PAHs), heavy metals, and estrogenic compounds (Sarkar et al., 2006). An increase in EROD activity has been documented in crab and clam species exposed to sediments containing high concentrations of polychlorinated biphenyls (PCBs) and PAHs (Martín-Díaz et al., 2007). The cytochrome P450 family has previously been detected by den Besten (1998) in the pyloric caeca and stomach of the sea star *A. rubens* as well as, the haemal plexus of the sea cucumber *Holothuria forskali*. Therefore, EROD activity is predicted to be the most prevalent in the digestive tissues (pyloric caeca, digestive gland, and intestine) of echinoderms.

Used lubricating oil (ULO), sometimes referred to as used/spent crankcase or engine oil, is a significant contributor to petroleum pollution due to consumption, which itself constitutes approximately 70% of the ~760 million litres of anthropogenic petroleum pollution released worldwide annually (NRC, 2003). Studies in Canada, the United States, and several other countries have found ULO to be a large contributor, if not the single largest source, of saturated and aromatic hydrocarbons in urban runoff (Environment Canada, 2003; Fam et al., 1987; Hoffman et al., 1982; Latimer et al., 1990), a primary input of pollutant into the aquatic environment. While acute sources of pollution (spills) are more publicized, long-term chronic petroleum pollution from human activities may be equally damaging in a more insidious way (Martinez-Gomez et al., 2010). As oil weathers, the lightweight volatile compounds evaporate, leaving larger molecules behind, including partially water-soluble PAHs (Galt et al., 1991). These compounds are of particular interest to researchers since they can accumulate in animals and cause biological damage (Bechmann et al., 2010). Through the use of water-accommodating fractions (WAFs) the effects of oil, which consists of a complex mixture of thousands of compounds (Singer et al., 2000), can be studied. WAFs contain the partially water-soluble compounds within oil that aquatic marine organisms are most likely to come in contact with (Gagnon and Holdway, 2000).

The present research was designed to develop biomarkers of oil pollution in marine invertebrates from temperate-cold and subarctic regions. Four species among the most common in subtidal areas of the Northwest Atlantic and Arctic Oceans were chosen: the predatory sea star *Asterias rubens*, the suspension-feeding brittle star *Ophiopholis aculeata*, the herbivorous omnivore sea urchin *Strongylocentrotus droebachiensis*, and the suspension-feeding sea cucumber *Cucumaria frondosa*. These exemplify four classes within the phylum Echinodermata; they also occupy different ecological niches and exhibit distinct reproductive strategies (Giese et al., 1991; Lawrence, 1987a; Mercier and Hamel, 2009). Two of them (*S. droebachiensis* and *C. frondosa*) are commercially exploited (Grabowski and Chen, 2004; Hamel and Mercier, 2008). The aim of the present study was twofold. (1) To ascertain whether there were seasonal and sex-related fluctuations in baseline levels of EROD and GPx activity within various tissues of the focal species, and (2) to test the sensitivity and specificity of standard enzymatic biomarkers through a series of acute exposures to WAF of ULO, by comparing induced responses to sex-dependent and seasonal fluctuations in baseline (control) activity levels.

2. Materials and methods

2.1. Species Collection

All species were collected from southeastern Newfoundland (47°18′57″N 52°48′37″W) between 10 and 20 m depth, inside a period of ~4 weeks at the onset of the study. Individuals were maintained until their use under flow-through conditions (2.5 L min^{-1}) in two large tanks (500 L) at a temperature of 6 °C and mirrored ambient photoperiod with a daytime light intensity of ~200 lx within the Joe Brown Aquatic Research Building (JBARB) of the Department of Ocean Sciences (Memorial University). For all species only adult individuals of similar sizes were chosen. Sea stars (n = 83 *A. rubens*, 8–11 cm radius) were fed ad libitum a diet of blue mussels (*Mytilus edulis*) while sea urchins (n = 113 *S. droebachiensis*, 8–10 cm test diameter) were fed kelp (*Laminaria* sp.), up to a week prior to experiments. The plankton feeders, such as sea cucumbers (n = 117 *C. frondosa*, 17–25 cm length) and brittle stars (n = 540 *O. aculeata*, 1.2–2.0 cm central disc diameter), had access to fine planktonic material available in the natural seawater supply to the tanks. Only healthy individuals that did not exhibit any lesions or signs of stress, such as blistering in sea cucumbers, loss of spines in sea urchins, or missing limbs in brittle stars and sea stars, were selected for the trials.

Sex was not a predetermined factor in this study; individuals were allocated to treatment groups at random. While most species display a 1:1 sex ratio, variances are not uncommon and, therefore, skewed sex ratios were encountered in some instances.

2.2. Experimental design and procedures

The experimental setup consisted of 8 independent replicate tanks (54 L) divided up between the 2 treatments (4 control and 4 experimental). Each tank hosted 2 sea stars, 2 sea urchins, 2 sea cucumbers, and 15 brittle stars. These tanks were submerged in larger flow-through tubs that maintained temperatures at 6 °C throughout the trials with supplementary air provided via bubblers. Each trial lasted 96-h under static conditions in order to minimize fluctuations in hydrocarbon concentration over time and maximize the amount of time individuals were exposed while maintaining oxygen concentration (Benson and Krause, 1984). All tanks were cleaned and maintained under flow-through conditions between trials (and the control and experimental tanks were never crossed). During the trials, 50% of the seawater and water-accommodating fraction (WAF) of the used lubricating oil (ULO), when applicable, was replaced every 24-h. Individuals were not