Aquatic Toxicology 92 (2009) 187–194

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Immune function in the Arctic Scallop, *Chlamys islandica*, following dispersed oil exposure

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ARTICLE INFO

Article history:
Received 1 October 2008
Received in revised form 19 January 2009
Accepted 21 January 2009

Keywords:
Arctic Scallop
Chlamys islandica
Biomarker
Phagocytosis
Oil
Immunotoxicity

ABSTRACT

With the current expansion of offshore oil activities in Arctic regions, there is an urgent need to establish the potential effects of oil-related compounds on Arctic organisms. As susceptibility to growth, disease and survival is determined partly by the condition of an organism's immune system, measurement of endpoints linked to the latter system provide important early warning signals of the sub-lethal effects of exposure to contaminants. This study assessed the impact of dispersed oil exposure on immune endpoints in the Arctic Scallop *Chlamys islandica*, using a combination of cellular and humoral biological responses. Laboratory exposures of *C. islandica* to sub-lethal dispersed oil concentrations (0.06 and 0.25 mg l^{-1}) were conducted over 15 days, followed by a 7-day recovery period in clean, filtered seawater. Cellular endpoints were significantly altered following dispersed oil exposure: haemocyte counts ($P < 0.01$) and protein levels ($P < 0.01$) were significantly elevated, whilst cell membrane stability ($P < 0.001$) and phagocytosis ($P < 0.01$) demonstrated a significant reduction. Whilst these results indicate alteration in the immune endpoints measured, this appears to be reversible upon removal of the contaminant stress. However, the impact of long-term continuous exposure and high-level acute exposure to oil is still unknown, and may have consequences for disease resistance and hence survival.

1. Introduction

Oil is a ubiquitous contaminant throughout the marine environment with at least 20,000 t $year^{-1}$ entering marine systems from offshore oil production alone (GESAMP, 2007). Although there has been rapid progress towards monitoring and assessing the impacts of oil on the coastal environment, most work has focused on the use of temperate species (Laffon et al., 2006; Francioni et al., 2007; Culbertson et al., 2008; Hylland et al., 2008). With the expansion of oil exploration in Arctic and Sub-Arctic regions, notably the Barents Sea and the North Alaskan Slope (AMAP, 1998), there is an urgent need to develop methods to assess the potential impact of oil on Arctic species. The ecological impact of oil in cold-water environments may vary from those observed in temperate and tropical regions as a result of environmental and biological factors characteristic of the Arctic environment. For example, the low temperatures of Arctic regions may alter the behaviour of oil, as oil dissolution is temperature dependent (Payne et al., 1991). Also, photochemical degradation of petroleum hydrocarbons is more restricted in the Arctic compared with temperate regions due to the former region experiencing <50% of the annual solar radiation received by temperate areas (AMAP, 1998). Finally, interaction with sea ice may not only alter oil dispersion, but oil trapped under an ice layer will have inhibited evaporation of volatile components, prolonging its toxicity (Engelhardt, 1994). These abiotic factors influence the distribution, composition and physical state of oil, which in turn determines the bioavailability of its components. Cold-water marine invertebrates also possess adaptations enabling survival at low temperatures; these include low respiration rates (Ahn and Shim, 1998), altered cell membrane composition (Gillis and Ballantyne, 1999) and increased antioxidant defences (Regoli et al., 2000). The combination of altered chemical behaviour of oil at low temperatures, together with the biological adaptations of cold-water species, may affect the susceptibility of Arctic organisms to oil-induced damage. In addition, biological recovery from such damage is predicted to be much slower than in temperate systems; a result of the low growth rates, higher generation turnover times and increased age at maturation that are characteristic of many Arctic organisms (AMAP, 1998).

It is difficult to extrapolate how oil will impact on Arctic organisms from what has been reported for temperate species. Whilst oil can be acutely toxic to marine organisms, disruptions in essential homeostatic mechanisms, such as the immune system, can also occur as a result of sub-lethal exposures (Pipe et al., 1999). Due

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doi:10.1016/j.aquatox.2009.01.010

to its complexity and integration with other physiological systems, the immune system is extremely vulnerable to xenobiotic stress (Galloway and Goven, 2006), and there is increasing evidence that numerous environmental contaminants impact immune function in a range of organisms (Galloway and Depledge, 2001; Auffret et al., 2002). A severely compromised immune system can result in rapid mortality, however, subtle changes in its function can be used as early warning indicators of environmental stress and, therefore, immune function is important in assessing the sub-lethal effects of contaminant exposure (Luengen et al., 2004).

Bivalves are good candidates for immunotoxicology studies and have been studied widely (Pipe et al., 1999; Canesi et al., 2003; Wootton et al., 2003b; Cartier et al., 2004; Gagnaire et al., 2004; Auffret, 2005; Ordás et al., 2007). The Arctic Scallop, *Chlamys islandica*, is the northernmost member of family Pectinidae distributed widely throughout the Sub-Arctic transitional zone of the North Atlantic (Thorarinsdóttir, 1993) and has been successfully used in previous biomarker studies focusing on oxidative stress (Camus et al., 2002). With a low metabolic rate, scallops accumulate pollutants in their tissues to an even greater extent than other bivalves, including the common sentinel species *Mytilus edulis* (Young-Lai and Aiken, 1986). The aim of this study was to determine the effect of dispersed oil exposure on the immune function of the Arctic Scallop *C. islandica*.

2. Materials and methods

2.1. Experimental design

2.1.1. Animal collection and handling

In March 2007, Arctic Scallops, *C. islandica* (80–100 mm shell length), were hand collected by divers from Porsanger Norway (70°1′N, 25°1′E), and transported by air to the exposure facility at IRIS-Biomiljø in Stavanger, Norway (58°57′N, 5°43′E). The scallops were packed prior to flying in a polystyrene fish box, lying between two layers of damp dense foam. They were met from the flight and transported directly to the lab (total transit time ~6 h). No mortality was recorded during transportation. Upon arrival, organisms were transferred to 600 l fibreglass tanks with a continuous flow (7.5 l min^{-1}) of 36 PSU filtered seawater (FSW) at 4 ± 1 °C. Scallops were maintained in these holding tanks and fed at regular intervals (Instant Algae® Shellfish Diet) for 6 weeks prior to experimentation.

2.1.2. Exposure setup

Organisms were exposed to dispersed Ekofisk crude oil in a continuous flow (4 l min^{-1}, checked daily) system (CFS), as described by Sanni et al. (1998). Scallops were exposed for up to 15 days; this exposure time was selected based upon earlier studies reporting significant sub-lethal effects in temperate species (*M. edulis*) following exposure to dispersed oil (Aas et al., 2002) and produced water (Hannam et al., 2009). The dispersed oil was carried through Teflon capillary tubing to a header tank generating a concentration of 5 ppm and from here delivered to individual exposure tanks using peristaltic pumps to achieve the required nominal concentrations. Scallops were divided between three 600 l fibreglass tanks (~60 per tank) comprising three treatments: FSW control, low (0.06 mg l^{-1}) and high (0.25 mg l^{-1}) dispersed oil. Based on the OSPAR discharge limit of 30 mg l^{-1} oil in produced water (OGP, 2006), the oil concentrations tested here (0.06 and 0.25 mg l^{-1}) represent 120–500× dilutions of the maximum permissible level of oil in PW, and are comparable to the observed effect concentrations reported form previous studies using dispersed oil exposures (Baussant, 2004; Larson, 2004). Exposures were maintained at 4 ± 0.5 °C and scallops fed daily using the microalgae concentrate, shellfish diet (Instant Algae®).

2.2. Chemical measurements

2.2.1. Seawater PAH analysis

Chemical (PAH) analyses were carried out on water samples taken from each exposure tank after 24 h and 7 days from the start of the exposure. Samples were collected in 2 l amber bottles containing hydrochloric acid (to maintain the pH < 2) and prepared for analysis within 48 h of collection. Based on the method described by Jonsson et al. (2004), the 16 PAHs on the USA EPA priority pollutant list were measured together with the alkyl homologues of naphthalene, chrysene, dibenzophiothene and phenanthrene/anthracene. Eight deuterated PAHs were added as quantitative internal standards (QIS) and mixed on a magnetic stirrer for 15 min prior to liquid–liquid extraction with 50 ml cyclohexane. Each extraction was carried out by stirring the sample and solvent on a magnetic stirrer for 30 min before pouring through a 2-l separating funnel. The water phase was drained back into the sampling flask and extracted a further two times. Combined extracts were dried with anyhydrous $NaSO_4$, and concentrated to 0.5 ml using a TurboVap 500 (Zymark Corporation, USA) and transferred to glass vials for analysis.

2.2.2. Tissue PAH analysis

Analysis of PAH body burden was conducted on whole tissue homogenate of three individuals from each treatment after 15 days exposure as described by Jonsson et al. (2004). An individual was opened and drained of seawater prior to dissecting out all soft tissue. Briefly, whole body tissue was macerated using cyclohexane-rinsed scissors and transferred to a glass vial pre-treated at 500 °C with Teflon Lock ©and stored at −80 °C. Before analysis, scallop tissue was weighed and three quantitative internal standards were added before saponification with methanolic sodium hydroxide under reflux (2 h). Digest was filtered and extracted three times with cyclohexane. Combined extracts were purified by normal-phase, solid-phase extraction, concentrated to 0.5 ml and stored in capped glass vials for analysis.

Water chemistry and biotic PAH analyses were conducted using Gas Chromatography (HP5890, Hewlett Packard, USA) connected to a Mass Spectrometer (Finnigan SSQ7000, USA) and analysed in selected ion monitoring mode (GC/MS-SIM).

2.3. Biological measurements

2.3.1. Haemolymph extraction

From each treatment, 10 scallops were sampled after 7 and 15 days exposure, and remaining scallops were transferred to clean FSW for 7 days. After this 7 days recovery period, a further 10 scallops were sampled from each treatment group. Haemolymph (approximately 0.8 ml) was extracted from the striated region of the posterior adductor muscle using a 21-gauge needle. This was then transferred to a siliconised Eppendorf® and stored on ice until analysis to minimise cell aggregation. All assays on haemolymph samples were conducted in triplicate.

2.3.2. Total haemocyte count (THC)

Immediately after extraction, sub-aliquots of haemolymph (20 μl) were diluted 1:3 with Baker's Formol Calcium (2% sodium chloride, 1% calcium acetate, 4% formaldehyde) to fix cells and prevent aggregation. Total haemocyte counts were then carried out using an improved Neubauer haemocytometer under 40× magnification.

2.3.3. Protein concentration

Protein concentration was determined using a modified microplate method (Bradford, 1976). Briefly, 5 μl diluted haemolymph samples [1:3 in physiological saline (0.02 M HEPES,