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Changes in physiological responses of an Antarctic fish, the emerald rock cod (*Trematomus bernacchii*), following exposure to polybrominated diphenyl ethers (PBDEs)

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ABSTRACT

Although polybrominated diphenyl ethers (PBDEs) have the ability to undergo long-range atmospheric transport to remote ecosystems like Antarctica, a recent study found evidence for a local source within the Antarctic. PBDEs from sewage treatment outfalls of McMurdo Station and Scott Base on Ross Island have been attributed to the high concentrations measured in emerald rock cod (*Trematomus bernacchii*). The potential impact of PBDEs on Antarctic fish physiology is unknown and therefore, the aim of this study was to obtain a greater understanding of physiological responses of emerald rock cod for assessing changes in ecosystem quality. A PBDE mixture (ΣPBDE 8 congeners) was administered fortnightly over 42 days and physiological changes were observed throughout this period and for a further 14 days thereafter. Changes in liver composition, molecular level changes and enzyme activities of selected detoxification-mediated and antioxidant defence markers were measured. Changes in total lipid, lipid peroxide and protein carbonyl concentrations in emerald rock cod liver were consistent with increases in nucleus surface area in the PBDE-treated groups, suggesting alterations in cellular function. Changes in the activities of selected antioxidant enzymes indirectly indicated oxidative stress, possibly resulting in the changes in liver composition. Additionally, glutathione-S-transferase (GST) activity reached its peak faster than that of ethoxyresorufin-O-deethylase (EROD), suggesting that during the early response to PBDE exposures there could be a greater involvement of GST-mediated detoxification. Thus, for at least the species examined here, protein carbonyl and lipid peroxides were useful and informative biomarkers for cellular level responses following PBDE-related exposure. Furthermore, our findings suggest that emerald rock cod exposed to PBDEs develop oxidative stress – a condition with potential consequences for fish growth, health and reproduction.

1. Introduction

Legacy pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls are ubiquitous in the Antarctic environment (Bargagli, 2008; Borghesi et al., 2008; Corsolini, 2009; Corsolini et al., 2006; Miller et al., 1999). More recently, despite waste treatment practices at McMurdo Station (Conlan et al., 2004) and Scott Base (Connor, 2008), a number of polybrominated diphenyl ether congeners (PBDEs) were detected in both the treated sewage sludge (total PBDE 4690 μg kg^{-1} dry weight) and in the sentinel species, emerald rock cod (*Trematomus bernacchii*) (total PBDE 1520 μg kg^{-1} lipid) in the vicinity of the McMurdo station outfall (Hale et al., 2008). Users of the Antarctic research stations, United States McMurdo Station and New Zealand Scott Base on Ross Island, Antarctica, regularly practised dumping of raw waste directly into the marine environment until the mid-1990s (Waterhouse, 2001). Treatment of raw waste was eventually put in place, to implement agreements from the Antarctic Treaty from 1959 that obliged all operators to minimise human impacts in Antarctica. Accordingly, all waste items other than sewage and domestic waste waters must be returned to the operators' home countries (Waterhouse, 2001). The use of technical PBDE mixtures,

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as fire preventative chemicals, in existing household items and appliances in the Antarctic research stations ensures long lasting releases into the marine environment; and yet the effects of such exposure on Antarctic fish is not known.

It is well known that the enzyme cytochrome P450 1A1 (cyp1a1) is involved in the metabolism of contaminants, such as PAHs, by activation of the aryl hydrocarbon receptor (AhR) in the aryl hydrocarbon pathway within the liver (Chen et al., 2001; Guengerich, 2008). Up-regulation of ethoxyresorufin-O-deethylase (EROD) activity, whose catalytic activity is used as a measurement of cyp1a1 induction (Whyte et al., 2000), has been used as a biomarker to indicate AhR-mediated toxicity (Guengerich, 2008). Glutathione-S-transferase (GST), a phase II metabolic enzyme involved in the conjugation of glutathione enzyme with contaminants, such as PAHs, protects cells against toxic effect and oxidative stress (van der Oost et al., 2003). Other responses to metabolism of contaminants in temperate fish include elevated levels of reactive oxygen species (ROS) (Carney Almroth et al., 2010; Parvez and Raisuddin, 2005), protein carbonyls (Almroth et al., 2005, 2008) and lipid peroxides (Oliveira et al., 2008; Sreejai and Jaya, 2010; Tellez-Bañuelos et al., 2009).

Changes in cyp1a1 enzyme activity following exposure to either individual or mixed PBDE congeners, or technical PBDEs mixtures, have been observed in temperate fish (Boon et al., 2002; Kuiper et al., 2004; Lebeuf et al., 2006; Raldúa et al., 2008), as well as in model test species, such as zebrafish (*Danio rerio*) (Kuiper et al., 2006) and fathead minnow (*Pimephales promelas*) (Sullivan et al., 2007). However, in one study involving Atlantic salmon (*Salmo salar*), PBDEs did not induce a cyp1a1 response (Boon et al., 2002); although a recent in vitro study on Atlantic salmon hepatocytes found that cyp1a was up-regulated (Søfteland et al., 2011). Overall, these studies indicate a variable cyp1a1 response to PBDE exposures across fish species. Fewer than a dozen contaminant exposure studies have been conducted on polar region fish (Benedetti et al., 2009, 2007; Lurman et al., 2010; Miller et al., 1999; Nahrgang et al., 2009, 2010) and none involved the use of PBDEs as the exposure contaminant. Thus, the effects of PBDE exposure on Antarctic fish cyp1a1 activities are unknown.

Studies on PBDE exposures have also been shown to cause oxidative stress in temperate fish (Raldúa et al., 2008; Shao et al., 2008). An in vitro study found that the PBDE congener BDE-47 causes oxidative damage to rainbow trout (*Oncorhynchus mykiss*) RT-W1 gill cells (Shao et al., 2010). However, it is not clear how polar region fish respond to conditions of oxidative stress. In emerald rock cod, the antioxidant enzymes catalase (CAT) and glutathione peroxidase (GPX) activities are not naturally enhanced (Benedetti et al., 2010) compared to notothenioid counterparts, suggesting this species may potentially be sensitive to stressors that influence ROS levels; change in the free radical scavenger enzyme Cu/Zn superoxide dismutase (Cu/Zn sod) activity during oxidative stress is not known in this species.

Antarctic fish have low basal metabolic rates resulting in reduced activity and thermal tolerance (Pörtner, 2006), as well as slow growth and low fecundity (Pörtner et al., 2008). Additionally, Antarctic fish have naturally high tissue oxygen concentrations, which result from their adaptive strategies to combat the oxygen-rich cold-water environment of Antarctica (Pörtner, 2002; Pörtner et al., 2007). To counteract the effect of high oxidative conditions (Pörtner, 2010) and in addition to the effects that low temperatures have on membrane fluidity (Hazel, 1995), Antarctic fish have evolved to incorporate high levels of poly-unsaturated fatty acids in cell membranes (Guderley, 2004), which are susceptible to lipid peroxidation by ROS (Pörtner, 2002). The result is increased levels and prolonged life of free radicals, arising in part from increased proton leakage that facilitates ROS formation (Abele and Puntarulo, 2004; Pörtner, 2002).

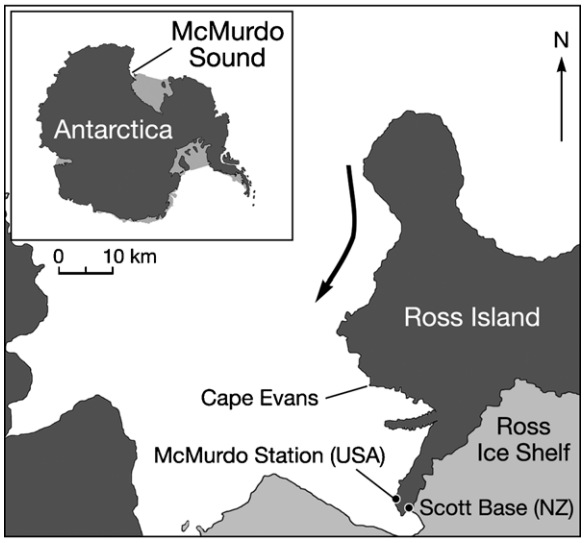

Fig. 1. Map of Antarctica (inset) and McMurdo Sound with the research stations, McMurdo Station and Scott Base, and with the site of emerald rock cod (*Trematomus bernacchii*) collection, Cape Evans. Arrow, from north to south, along the west-coast of Ross Island indicates the direction of water-current.

It is not known how Antarctic fish may respond to PBDE exposures that have the potential to enhance ROS levels. The objective of this present study was to investigate the physiological responses of emerald rock cod to oral exposure of a mixture of PBDEs over a 42-day period. Effects were measured using changes in fish condition and changes in the liver compositions of emerald rock cod (relative liver size, nucleus surface area, total lipids, lipid peroxides and protein carbonyls) as well as changes in mRNA copy numbers and enzyme activities of some detoxification-mediated and antioxidant defence markers (cyp1a1, EROD, GST, Cu/Zn sod, CAT and GPX). The end goals of this study were to (a) increase our understanding of the physiological effects that pollution has on polar species with cold-adapted physiologies, such as the emerald rock cod, which may be useful for developing mitigation measures and (b) identify suitable markers that may be useful for recognizing PBDE exposure.

2. Materials and methods

2.1. Animal collection

During October 2009, live emerald rock cod were captured after drilling through first year sea-ice at Cape Evans, Antarctica (77°38′S, 166°24′E) (Fig. 1). Fish collected from this location, which is approximately 20 km upstream (in terms of ocean currents) from the research stations, was assumed to be negligibly affected by pollution from the research stations. Fish were brought up to the surface using hook and line with artificial lures and then transported in insulated boxes, filled with seawater, to Scott Base. Within two weeks of capture, all fish were transported to a refrigerated unit at the University of Canterbury, Christchurch, New Zealand for the dose–response study (further information on animal transport can be found in supplementary material). To the best of our ability, only sexually immature fish (identified as having <230 mm body length) were collected since sampling occurred during the spawning season (La Mesa et al., 2006).

One fish died during the 5-h plane trip from Ross Island (Antarctica) to Christchurch (New Zealand). Another individual was identified as a gravid female during the acclimatisation period at the quarantine facilities (Christchurch, New Zealand) and was donated to the International Antarctic Centre (Christchurch, New Zealand). There were no additional mortalities during the study.