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Assessing Aromatic Hydrocarbon Exposure in Antarctic Fish Captured Near Palmer and McMurdo Stations, Antarctica

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Abstract. Since little is known about the effects of contaminants on Antarctic organisms, the effects of polynuclear aromatic hydrocarbons (PAH) on Antarctic fish were evaluated. Fish captured near Palmer Station on the Antarctic Peninsula exhibited induced ethoxyresorufin O-deethylase (EROD) activities and elevated concentrations of biliary PAH metabolites compared to fish from control sites. Naphthalene and phenanthrene PAH metabolite levels were significantly higher in the bile of fish captured near McMurdo Station than in fish from remote sites in McMurdo Sound. Laboratory experiments were conducted in which *Notothenia gibberifrons* were treated with benzo[a]pyrene (BaP) and diesel fuel Arctic (DFA). Although DFA is composed primarily of 2- and 3-ring PAH which are not known to be potent CYPIA inducers, the maximal hepatic EROD activity of DFA-treated fish was approximately 80% of that observed in BaP-treated fish. Additionally, 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic equivalents (TEQs) were determined for hepatic extracts of laboratory-dosed and field-captured fish using rat hepatoma H4IIE cell bioassays. The TEQ values of H4IIE cells dosed with hepatic extracts of DFA-treated fish correlated more closely with hepatic concentrations of 3-ring and >3-ring PAH than with 2-ring and ΣPAH concentrations. However, bioassay-derived TEQs were higher than expected based on the measured levels of ≥3-ring PAH in the hepatic extracts of DFA-treated fish. The TEQs for hepatic tissue extracts of BaP-treated fish paralleled tissue concentrations of PAH. The TEQs for field captured fish were significantly lower than those derived from dosed fish extracts.

Environmental processes have been studied for more than thirty years in polar environments and numerous studies have documented the presence of chemical contaminants in Antarctica. The major sources of contaminants to the Antarctic environment are global atmospheric transportation and localized activities related to human settlements (Kennicutt *et al.* 1995; Lenihan *et al.* 1990; Risebrough *et al.* 1990). The most widespread contaminants in Antarctica are petroleum-related. Although the presence of contaminants in the Antarctic environment has been established, few studies document their effects on indigenous fauna.

Hydrocarbon contamination has been reported in sediments and in the tissues of fish and invertebrates collected in waters near Palmer Station (Kennicutt *et al.* 1992a, 1992b) and McMurdo Station (Lenihan *et al.* 1990). Palmer Station is a relatively small facility (10-60 inhabitants) located adjacent to Arthur Harbor on the Antarctic Peninsula. McMurdo Station is located off McMurdo Sound on the Antarctic Continent and can support over 1200 people. In Arthur Harbor, sediment hydrocarbon contamination was traced to fuel spills; ship and boating activities; runoff from Palmer Station; and the grounding of an Argentine supply ship in 1989 (Kennicutt *et al.* 1991, 1992a). Other studies documented an intense contaminant gradient within Winter Quarters Bay (WQB) near McMurdo Station in which high concentrations of polynuclear aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCBs) were measured (Kennicutt *et al.* 1995; Lenihan *et al.* 1990; Risebrough *et al.* 1990). Contamination has been linked to an inactive dump site; fuel storage tanks; shipping and construction activities; station runoff; and past disposal practices.

Historically, the availability of contaminants was assessed by measuring the amount of contaminants sequestered in biological tissues. However, in recent years, various *in vitro* and *in vivo* biomarkers have been used to evaluate exposure (Ankley *et al.* 1991; Krahn *et al.* 1992; Payne *et al.* 1987; Stegeman and Lech 1991; Stegeman *et al.* 1987; Tillitt *et al.* 1991a; Van Veld *et al.* 1990). The most commonly used indicators of hydrocarbon exposure are cytochrome P450 induction responses. A P450 (CYPIA) has been identified in teleost fish that is similar to mammalian CYPIAI (Heilmann *et al.* 1988) and can be induced by exposure to selected organic contaminants (Collier and Varanasi 1991; Stegeman *et al.* 1987; Van Veld *et al.* 1990). Hydrocarbon exposure in Antarctic fish was assessed by measuring biliary PAH metabolite concentrations and hepatic ethoxyresorufin O-deethylase (EROD) activity. Hepatic PAH metabolites are concentrated in bile for elimination (Varanasi *et al.* 1989) and levels of fluorescent aromatic metabolites are

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correlated with PAH exposure in fish (Collier and Varanasi 1991; Krahn *et al.* 1984, 1992; Varanasi and Gmur 1981). EROD activity is catalyzed by CYPIA and this response is sensitive to organic xenobiotics and can be used to evaluate exposure (Stegeman and Lech 1991).

Laboratory studies were also conducted in which Antarctic fish were dosed with diesel fuel Arctic (DFA) and benzo[a]pyrene (BaP) to determine their aryl hydrocarbon (Ah) receptor responsiveness. Additionally, an *in vitro* assay in which the induction of EROD activity in rat hepatoma H4IIE cells exposed to liver extracts from both field and laboratory dosed-fish was used to determine 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalents (TEQs). *In vitro* rat hepatoma H4IIE cell bioassays were developed to determine the biological potency of extracts from a variety of environmental and industrial samples which contain TCDD-like activity (Bradlaw and Casterline 1979; Trotter *et al.* 1982; Zacharewski *et al.* 1989). Since TCDD-like compounds elicit similar toxic and biochemical responses via the Ah receptor signal transduction pathway (Safe 1990), various Ah receptor-mediated responses including P450IAI induction have been used to determine bioassay-derived TEQ values for mixtures of chemicals (Ankley *et al.* 1991; Bradlaw and Casterline 1979; Tillitt *et al.* 1991a, 1991b; Trotter *et al.* 1982). This approach detects all bioactive components in a mixture and assesses responses to coextracted non-TCDD-like compounds.

Materials and Methods

Field Collections

Notothenia coriiceps neglecta and *N. gibberifrons* were captured at Low Island and in Dallman Bay using a 5.5 m otter trawl on the R/V *Polar Duke* in March and April 1991, 1992, and 1993 (Figures 1(a), 1(b)). Fish were transported to Palmer Station in on-board flow-through sea water tanks. *N. coriiceps neglecta* was captured in Arthur Harbor and near the *Bahía Paraiso* wreckage using traps and hook and line in March and April 1991, 1992, and 1993 (Figures 1(c), 1(d)). Sediments were collected in 1991 by divers around Palmer Station and in the vicinity of the *Bahía Paraiso* wreck. In McMurdo Sound, fish *(Trematomus bernacchii)* and sediments were collected by divers in 1991 and 1992 from contaminated sites near McMurdo Station and control sites in McMurdo Sound (Figures 1(e), 1(f)). Fish were dissected immediately after capture and bile and liver samples (for PAH analyses and *in vitro* assays) were frozen to −20°C until analysis.

Laboratory Fish Experiments

N. gibberifrons were maintained in 1.7 m or 2.7 m diameter flow-through seawater tanks at Palmer Station at 0 ± 1°C. Fish were held for 7–8 days and were not fed during captivity. Fish were injected intramuscularly with either 500 μL of DFA, which based on ΣPAH concentration was equivalent to a dosage of 29 mg · kg^{-1}, or approximately 5 mg · kg^{-1} BaP in 2 mL · kg^{-1} acetone. Control fish were injected with 2 mL kg^{-1} of acetone. Fish were sacrificed at 6, 12, 24, 48, 72, 96, 120, and 144 h after injection. Four individual fish were analyzed for EROD activity, biliary metabolite levels were measured on 5–8 individuals, and tissue from four fish was pooled for PAH analysis.

Tissue PAH Analysis

Briefly, tissues were macerated and extracted three times in CH_2Cl_2 with a tissuemizer. The CH_2Cl_2 aliquots were combined and concentrated to 2 mL in hexane. The concentrated extract was then purified by alumina/silica gel or alumina/sodium sulfate column chromatography followed by high performance liquid chromatography (HPLC). Extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode. Details of methods and instrument conditions are described elsewhere (Wade *et al.* 1993). The GC/MS was calibrated and linearity was determined by injection of standards at five concentrations. Peak identity was confirmed by molecular ion, the ratio of the primary (base) ion to the secondary ion, and retention time. Instrument calibration was checked daily by injection of a calibration mixture. The calibration check was maintained to within ± 15% on average for all analytes of interest. Quality assurance for each set of 10 samples included a system blank, a matrix spike, and a matrix spike duplicate, which were carried through the entire analytical scheme in a manner identical with the samples. Surrogates were added to all samples prior to extraction. Data are reported as ΣPAH ng · g^{-1} dry weight. ΣPAH values were determined by summing the following parent analytes that were detected and those alkylated analytes whose concentrations were >1/2 method detection limit (MDL): naphthalene; C_1-C_4 naphthalenes; biphenyl; acenaphthylene; acenaphthene; fluorene; C_1-C_3 fluorenes; phenanthrene; anthracene; C_1-C_4 phenanthrenes/anthracenes; dibenzothiophene; C_1-C_3 dibenzothiophenes; fluoranthene; pyrene; C_1 fluoranthenes/pyrenes; chrysene; benzo[a]anthracene; C_1-C_4 chrysenes/benzo[a]anthracenes; benzo[b]fluoranthene; benzo[k]fluoranthene; benzo[a]pyrene; benzo[e]pyrene; indeno-[1,2,3-cd]pyrene; dibenz[ah]anthracene; and benzo[ghi]perylene. MDLs were calculated by the method of Keith *et al.* (1983).

Sediment PAH Analysis

Sediments were freeze-dried and Soxhlet extracted in CH_2Cl_2. After extraction, samples were purified by alumina/silica gel chromatography. Aromatic hydrocarbons were determined by GC/MS in selection ion monitoring mode. Details of methods and instrument conditions are described elsewhere (Wade *et al.* 1993). The GC/MS was calibrated and linearity was determined by injection of standards at five concentrations. Peak identity was confirmed by molecular ion, the ratio of the primary (base) ion to the secondary ion, and retention time. Instrument calibration was checked daily by injection of a calibration mixture. The calibration check was maintained to within ± 15% on average for all analytes of interest. Quality assurance for each set of 10 samples included a system blank, a matrix spike, and a matrix spike duplicate which were carried through the entire analytical scheme in a manner identical with the samples. Surrogates were added to all samples prior to extraction. Data are reported as ΣPAH ng · g^{-1} dry weight. ΣPAH values were determined by summing the following parent analytes that were detected and those alkylated analytes whose concentrations were >1/2 method detection limit (MDL): naphthalene; C_1-C_4 naphthalenes; biphenyl; acenaphthylene; acenaphthene; fluorene; C_1-C_3 fluorenes; phenanthrene; anthracene; C_1-C_4 phenanthrenes/anthracenes; dibenzothiophene; C_1-C_3 dibenzothiophenes; fluoranthene; pyrene; C_1 fluoranthenes/pyrenes; chrysene; benzo[a]anthracene; C_1-C_4 chrysenes/benzo[a]anthracenes; benzo[b]fluoranthene; benzo[k]fluoranthene; benzo[a]pyrene; benzo[e]pyrene; indeno-[1,2,3-cd]pyrene; dibenz[ah]anthracene; and benzo[ghi]perylene. MDLs were calculated by the method of Keith *et al.* (1983).

HPLC/Fluorescence Screening of Bile

Bile was analyzed by an HPLC/fluorescence detection method described in Krahn *et al.* (1984). Aromatic compounds fluorescing at naphthalene (290/335), phenanthrene (260/380), and benzo[a]pyrene (380/430) excitation/emission wavelengths were analyzed. Metabolites eluting from the column within specific retention times were summed to yield total fluorescence base on equivalents of known

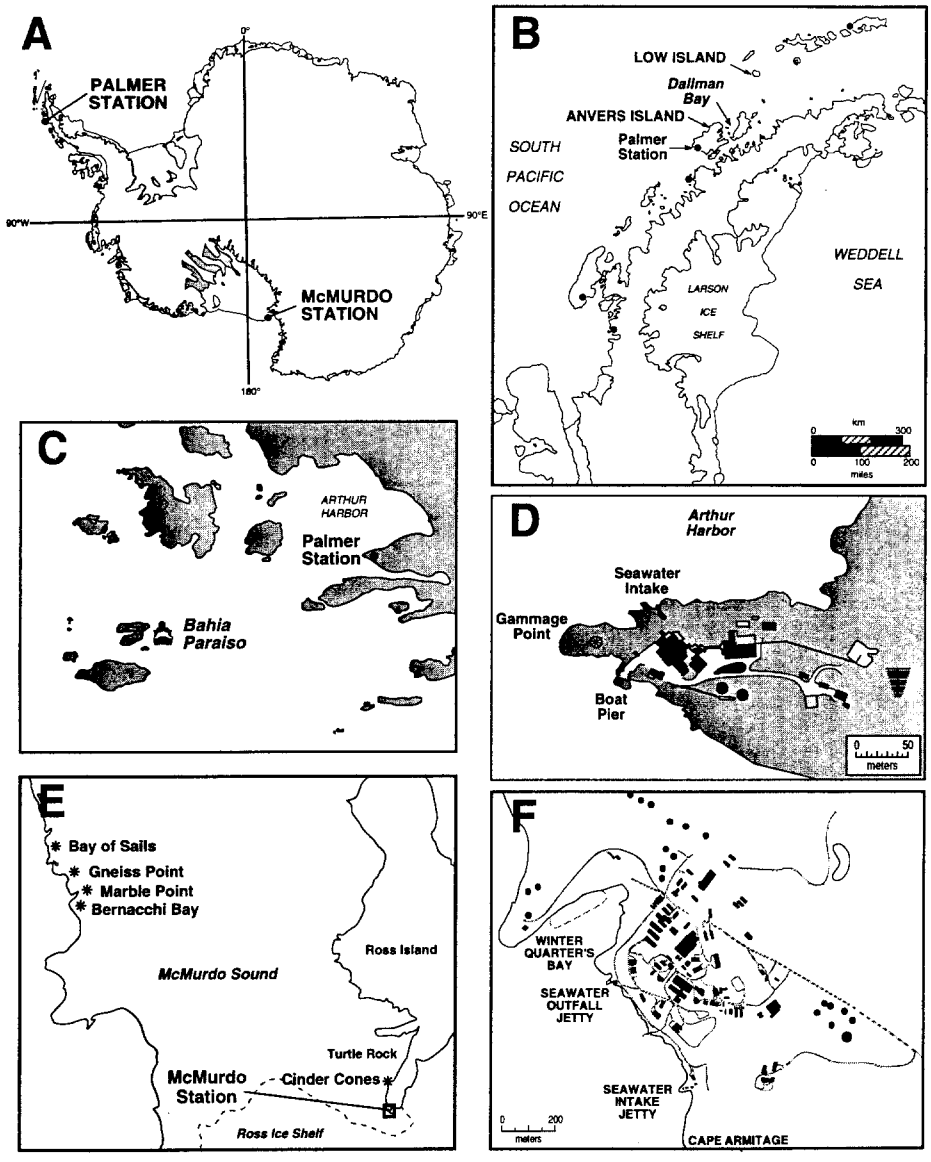

Fig. 1. Location of Palmer Station and McMurdo Station on the Antarctic continent (A), location of collection sites near the Antarctic Peninsula (B), location of Arthur Harbor and the *Bahía Paraiso* wreckage near Palmer Station, Antarctica (C), location of collection sites around Palmer Station, Antarctica (D), location of collection sites in McMurdo Sound, Antarctica (E), location of collection sites around McMurdo Station, Antarctica (F)

amounts of naphthalene, phenanthrene, and benzo[a]pyrene standards. Quality assurance for each set of 10 samples included an instrument blank and sample duplicate. Additionally, instrument calibration was verified daily, using a pooled reference bile sample.

EROD Activity

Microsomes were prepared by differential centrifugation from fresh livers using modifications of a protocol described by Stegeman *et al.* (1987). Livers were homogenized in a 0.1M Tris, pH 7.5 buffer containing 0.25M sucrose. After centrifugation, microsomes were stored in liquid nitrogen in a 50mM Tris, pH 7.5 buffer containing 1mM EDTA, 1mM DTT, and 20% glycerol until analysis. Protein was determined by the bicinchoninic acid (BCA) protein determination method (Smith *et al.* 1985). EROD activity was measured on microsomal preparations. The reaction mixture consisted of 0.1M HEPES, pH 8.0, 0.7 mg NADPH, 0.7 mg NADH, 0.7 mg BSA, 0.7 mg $MgCl_2$, and 100-250 μg microsomal protein. Reactions were initiated by the addition of 50 μL of 100 mM 7-ethoxyresorufin and proceeded at 30°C for 15 min. The reaction was terminated by the addition of 2.5 mL of methanol and read on a spectrofluorometer at 500 nm excitation and 585 nm emission wavelength settings. All samples and method blanks were analyzed in duplicate for each sample set.

In Vitro *Bioassay*

Liver extracts used in the bioassays were obtained by a method identical to that used to extract PAH from tissues except that analytical surrogates were not added and the extracts were concentrated and dissolved in dimethyl sulfoxide (DMSO). H4IIE cells were grown as continuous cell lines in α-essential medium supplemented with 2.2 mg $\cdot$ mL^{-1} tissue culture grade sodium bicarbonate, 5% fetal calf serum, and 10 mL $\cdot$ L^{-1} antibiotic-antimycotic solution. Stock cultures were grown in 150 cm^2 tissue culture flasks and incubated in a humidified mixture of 5% CO_2 and 95% air under atmospheric pressure. For enzyme assays, approximately 3×10^4 cells in 5 mL of media were passaged to 25 cm^2 tissue culture flasks. Solutions of the liver extracts dissolved in DMSO were added to the culture flasks so that the final concentration of DMSO in the medium was $< 1.0\%$. Cells were harvested by manual scraping from the culture flasks, centrifuged at 1000 X g for 6 min at 4°C, and resuspended in 100 μL of Tris-sucrose buffer (38 mM Tris-HCl, 0.2 M sucrose, pH 8.0). Aliquots (50 μL) of

Table 1. Hepatic total polynuclear aromatic hydrocarbon (ΣPAH) concentrations and biliary PAH metabolite concentrations of *Notothenia coriiceps neglecta* collected near the Antarctic Peninsula

	Sample type		
	Bile	Bile	Liver
Site	Naphthalene ng · g^{-1}	Phenanthrene ng · g^{-1}	ΣPAH ng · g^{-1}
Bahía Paraiso	69,000 ± 56,000 (n = 22)	9,400 ± 9,900 (n = 22)	569 ± 500 (n = 10)
Palmer Station	77,000 ± 46,000 (n = 21)	11,000 ± 8,300 (n = 21)	398 ± 189 (n = 12)
Dallman Bay and Low Island	33,000 ± 14,000 (n = 13)	5,100 ± 2,000 (n = 13)	268 ± 142 (n = 8)

the cell suspension were incubated with 1.15 mg $MgSO_4$ in 0.1 M HEPES buffer, pH 7.5) in a 37°C water bath for 2 min. The reaction was started by adding 50 μL ethoxyresorufin solution (1 mg · 40 mL^{-1} methanol) for a 15 min incubation and stopped by adding 2.5 mL methanol. Samples were centrifuged at 1000 × g for 10 min. The supernatant was used for fluorescence measurement at an excitation wavelength of 550 nm and emission wavelength of 585 nm (Pohl and Fouts 1980). Samples were run in triplicate and the results are expressed as means ± standard deviation.

TCDD-induced EROD activity was used as a positive control and 1 nM TCDD (0.644 ng/plate) was used to determine the 100% maximal response. Since the dose response curve for induction of EROD activity in rat hepatoma cells by TCDD was nearly linear from 0 to 1 nM, the TCDD TEQ was determined for the extracts. A method blank and duplicate sample were extracted for each set of 10 or fewer samples.

Table 2. Hepatic ethoxyresorufin O-deethylase (EROD) activity of *Notothenia coriiceps neglecta* captured near the Antarctic Peninsula

	EROD
Site	nmol · min^{-1} · mg^{-1}
Palmer Station	
Boat pier	0.121 ± 0.54 (n = 4)
Seawater intake	0.030 ± 0.020 (n = 11)
Bahía Paraiso wreck	0.030 ± 0.029 (n = 6)
Remote sites	0.001 ± 0.002 (n = 4)

Statistics

Data were analyzed using analysis of variance (ANOVA) on ranked data or regression analysis. Level of significance was $p \leq 0.05$. All non-detected data were coded as 0 for statistical purposes.

Results

Antarctic Peninsula

Dallman Bay and Low Island are remote from known human activities and served as control sites for the Peninsula studies (Figure 1(b)). ΣPAH concentrations were highly variable in sediments collected in Arthur Harbor, ranging from 201–14,490 ng · g^{-1}. The highest concentrations were measured in sediments collected in the subtidal zone close to Palmer Station in the boat basin and near Gammage Point (Figure 1(d)). Elevated sediment PAH concentrations were also measured adjacent to the *Bahía Paraiso* wreckage. PAH with four or more condensed aromatic rings were typically below the method detection limits (~5 ng · g^{-1}). There was no significant difference in ΣPAH concentrations in the livers of *N. coriiceps* captured at any of the Peninsula sites (Table 1). However, a range of total PAH concentrations (50-1180 ng · g^{-1}) was measured in fish captured near the *Bahía Paraiso* wreckage.

The concentrations of biliary PAH metabolites were significantly higher in *N. coriiceps neglecta* captured near Palmer Station and adjacent to the *Bahía Paraiso* wreckage than those measured in fish captured at remote sites (Table 1). BaP equivalent metabolites in fish captured near the Antarctic Peninsula were typically below the method detection limit (< 100 ng · g^{-1}) and thus the data are not presented.

Hepatic EROD activities in *N. coriiceps neglecta* captured at the remote sites were low to non-detectable (Table 2). The highest EROD activities were measured in fish captured near docking facilities at Palmer Station (Figure 1(d)). EROD activities were significantly lower in fish caught near the seawater intake at Palmer Station and near the *Bahía Paraiso* wreckage.

McMurdo Station

The highest sediment ΣPAH concentrations in McMurdo Sound were measured in WQB (Figure 2). Levels in WQB ranged from 687–6339 ng · g^{-1}. Significant levels of ≥ 4-ring PAH (56–612 ng · g^{-1}) were also detected in WQB sediments (Figure 2). Sediment ≥ 4-ring and ΣPAH concentrations were significantly lower near Cape Armitage (~1 km from WQB) and at the remote sites (Figure 1(e)). Fish were collected in WQB, at Cape Armitage, and at Cinder Cones, a site distant from McMurdo Station. Due to the limited number and size of these fish, all of the soft visera was used for contaminant analysis. The highest tissue PAH concentrations were measured in fish collected in WQB. Although ΣPAH levels were

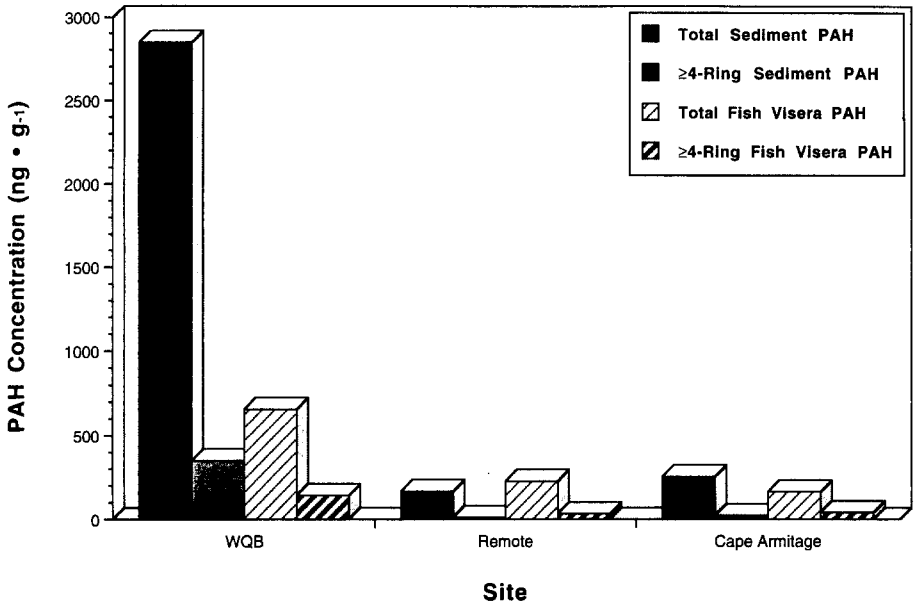

Fig. 2. Total PAH and ≥4-ring PAH concentrations in sediments and fish tissues from McMurdo Sound, Antarctica. Total Sediment PAH = sum of all PAH in sediments in ng · g^{-1}; ≥4-Ring Sediment PAH = sum of all 4 ring or greater PAH in sediments in ng · g^{-1}; Total Fish Visera PAH = sum of all PAH in the visera of fish in ng · g^{-1}; ≥4-Ring Fish Visera PAH = sum of all 4 ring or greater PAH in the visera of fish in ng · g^{-1}. Sediments were collected in Winter Quarters Bay (WQB), remote (Bay of Sails, Bernacchi Bay, Marble Point, and Gneiss Point), and Cape Armitage. Fish were collected in Winter Quarters Bay (WQB), Cape Armitage, and remote (Cinder Cones)

Table 3. Biliary polynuclear aromatic hydrocarbon (PAH) metabolite concentrations of fish collected in McMurdo Sound

		Fluorescence ng · g^{-1}		
Site	Species	Naphthalene	Phenanthrene	BaP
Winters Quarters Bay	*Trematomus bernacchii*	140000 ± 51000 (n = 18)	25000 ± 13000 (n = 18)	940 ± 730 (n = 18)
Cape Armitage	*Trematomus bernacchii*	92000 ± 36000 (n = 4)	13000 ± 5100 (n = 4)	930 ± 440 (n = 4)
Cinder Cones	*Trematomus bernacchii*	51000 ± 27000 (n = 16)	7200 ± 3700 (n = 16)	780 ± 430 (n = 16)

significantly lower in fish captured at Cinder Cones and Cape Armitage, there was no significant difference in the concentrations of ≥ 4-ring PAH.

Biliary concentrations of PAH metabolites were also determined in *T. bernacchii* collected in McMurdo Sound. The highest levels of naphthalene and phenanthrene metabolites were measured in fish collected in WQB and were significantly lower in fish captured at both Cape Armitage and Cinder Cones (Table 3). However, there was no significant difference in biliary BaP concentrations for the three sites (Table 3).

Laboratory Studies

N. gibberfrons were dosed with either DFA or BaP. Hepatic levels of PAH, EROD activity, and biliary PAH metabolite concentrations were measured during the course of the experiment.

Total PAH levels in the livers of DFA-dosed fish reached maximal levels within 24-48 hours (Figure 3). Greater than 90% of the total hepatic PAH levels were due to 2-ring PAH (primarily naphthalenes, Figure 3). Three-ring (primarily anthracenes and phenanthrenes) and >3-ring PAH (primarily fluoranthene, pyrene, benzo[a]anthracene, chrysenes, benzofluoranthenes, and benzopyrenes) concentrations were low. Maximum tissue levels of 3 ring or larger PAH were measured 72 h after administration. Total hepatic PAH concentration (>99% BaP) in BaP-dosed fish were variable with time (Figure 4) and maximum levels were observed after only six hours.

Hepatic EROD activities were higher in fish dosed with BaP than DFA for most time intervals (Table 4). BaP dosed fish exhibited maximal EROD activity between 96 and 144 h after injection, whereas maximal EROD activity was observed at 144 h in DFA-treated fish.

In Vitro *Assays*

The induction of TCDD TEQs in rat hepatoma H4IIE cells by extracts from livers of fish dosed with DFA and BaP is presented in Figures 3 and 4. For DFA-dosed fish, the maximal TEQ was observed after 72 hours. TEQs were highest in fish 12 and 72 h after treatment with BaP (Figure 4). In both experiments, TEQs declined markedly between 24 and 48 hours. TEQ induction in H4IIE cells dosed with aliquots of liver extracts from BaP-dosed fish parallels hepatic BaP concentrations (Figure 4). TEQ induction in rat hepatoma cells exposed to hepatic extracts from DFA-dosed fish correlated most closely with 3-ring and >3-ring PAH concentrations (Figure 3).

Rat hepatoma H4IIE cells were also treated with extracts from livers of *Notothenia coriiceps neglecta* captured near

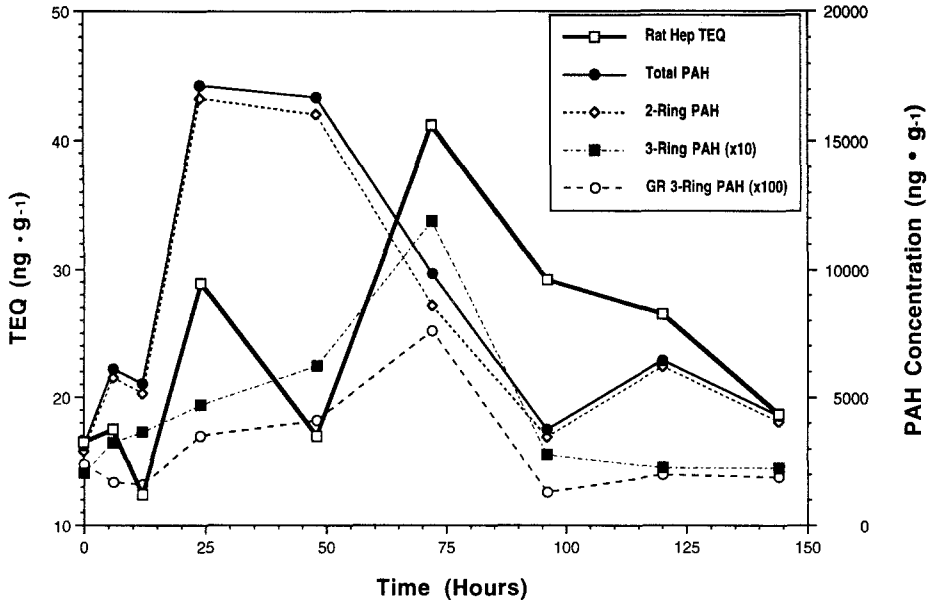

Fig. 3. The relationship between PAH concentrations in the livers of fish dosed with DFA and TEQs derived from the induction of EROD activity in rat hepatoma H4IIE cells exposed to liver extracts of fish dosed with DFA. Rat hep TEQ = TCDD TEQs (ng · g^{-1}) of H4IIE cells exposed to the liver extracts of dosed fish; Total PAH = sum of all PAH in livers in ng · g^{-1}; 2-Ring PAH = sum of all 2 ring PAH in livers in ng · g^{-1}; 3-Ring (× 10) = sum of all 3 ring PAH × 10 in livers in ng · g^{-1}; GR 3-Ring PAH (× 100) = sum of all PAH containing more than 3 rings × 100 in livers in ng · g^{-1}. Fish were sacrificed at 0, 6, 12, 24, 48, 72, 96, 120, and 144 hours after injection. An aliquot of liver extract was analyzed for PAH concentrations and H4IIE cells were dosed with another aliquot of liver extract at each time point

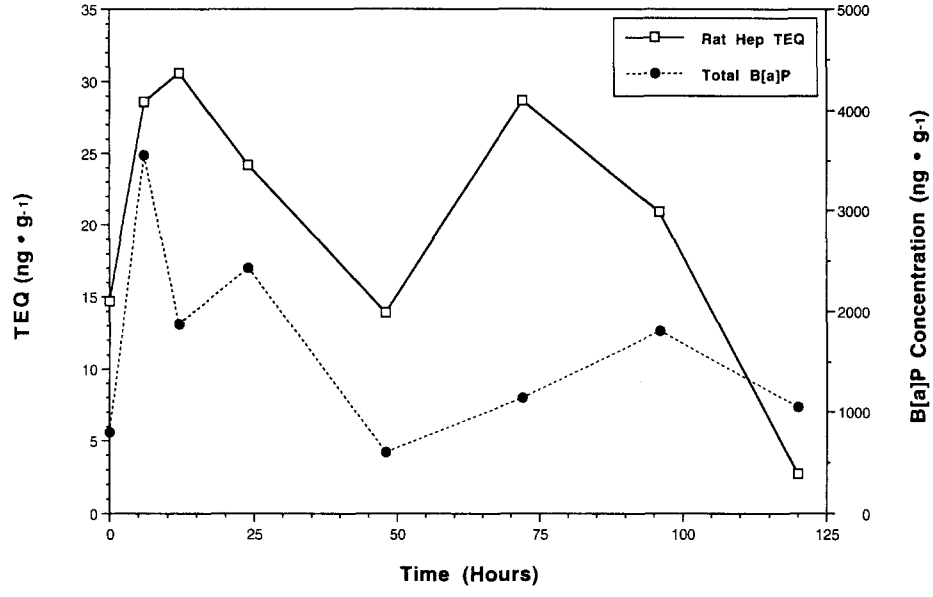

Fig. 4. The relationship between PAH concentrations in the livers of fish dosed with BaP and TEQs derived from the induction of EROD activity in rat hepatoma H4IIE cells exposed to liver extracts of fish dosed with BaP. Rat hep TEQ = TCDD TEQs activity (ng · g^{-1}) of H4IIE cell exposed to the liver extracts of dosed fish; Total B[a]P = the concentration of BaP in livers in ng · g^{-1}. Fish were sacrificed at 0, 6, 12, 24, 48, 72, 96, 120, and 144 hours after injection. An aliquot of liver extract was analyzed for PAH concentrations and H4IIE cells were dosed with another aliquot of liver extract at each time point

Palmer Station and the *Bahía Paraiso* wreckage and *Notothenia gibberifrons* collected at control sites. EROD activity was only induced by extracts from 2 fish captured near Palmer Station and the TEQs were substantially lower (0.06 ± 1.1 ng · g^{-1}) than those for dosed fish.

Discussion

Few studies have documented the biological effects associated with contaminant exposure in Antarctica. Limpet and bird mortalities were observed in Arthur Harbor after the grounding of the *Bahía Paraiso* released approximately 600,000 L of diesel fuel (Eppley and Rubega 1990; Kennicutt *et al.* 1990). In McMurdo Sound, dramatic changes in benthic invertebrate communities were correlated with a hydrocarbon contaminant gradient (Lenihan *et al.* 1990). This study provides evidence for PAH exposure in Antarctic fish captured near Palmer and McMurdo Stations.

Localized, elevated biliary naphthalene and phenanthrene equivalent metabolite concentrations and induced hepatic EROD activities were observed in fish from Arthur Harbor. These enhanced concentrations/activities correlated with sediment contaminant levels of ΣPAH. Subtidal sediment PAH concentrations in Arthur Harbor are typically at background levels with few values elevated above background (Kennicutt *et al.* 1992a). Elevated PAH concentrations are found in sediments adjacent to Palmer Station and the *Bahía Paraiso* wreckage. The highest concentrations of biliary metabolites and *in vivo* hepatic EROD activities were in fish collected between the boat basin and Gammage Point. The boat basin is the site of small boat operations and Gammage Point is the dock for larger vessels. Both areas are subject to diesel fuel spillage associated with fueling, maintenance, and transportation activities. PAH

Table 4. Time-dependent induction of hepatic ethoxyresorufin O-deethylase (EROD) activities from *Notothenia gibberifrons* dosed with DFA and BaP

	Treatment	
Time (h)	DFA nmol · min^{-1} · mg^{-1}	BaP nmol · min^{-1} · mg^{-1}
0	0.093 ± 0.023	ND[a]
24	0.060 ± 0.018	0.185 ± 0.057
48	0.219 ± 0.090	0.400[b] ± 0.157
96	0.732[b] ± 0.136	1.518[b] ± 0.347
144	1.267[b] ± 0.136	1.614[b] ± 0.416

[a] not detected
[b] significantly higher than observed in control fish (Time 0)

levels are lower in sediments near the seawater intake for Palmer Station, a short distance from Gammage Point and the boat basin. Fish collected near the seawater intake had significantly lower EROD activities than fish captured between the boat basin and Gammage Point. Although the *Bahía Paraiso* ran aground in 1989, fish collected near the wreckage in 1993 still exhibited higher levels of PAH metabolites and EROD activity than those from remote sites, indicating that PAH contamination continues. However, there were no significant differences in the concentration of PAH in the livers of fish collected in Arthur Harbor and remote sites. In general, tissue levels of PAH do not accurately reflect PAH exposure because fish efficiently metabolize PAH and consequently only trace quantities of the parent compounds are detected in tissues (Varanasi *et al.* 1989).

BaP equivalent metabolites were below detection limits for fish captured along the Antarctic peninsula, which is consistent with the low or non-detectable levels of ≥ 4-ring PAH in sediments and tissues from Arthur Harbor (Kennicutt *et al.* 1991, 1992a, 1992b, 1995). Previous studies showed that DFA is the primary source of aromatic hydrocarbon contamination in Arthur Harbor (Kennicutt *et al.* 1992a). Additionally individual metabolites of alkylated naphthalenes, phenanthrenes, and dibenzothiophenes were the prominent metabolites in the bile of fish captured near the *Bahía Paraiso* and Palmer Station, which also indicates that fish are primarily exposed to the lower molecular weight PAH characteristic of DFA (McDonald *et al.* 1992).

Naphthalene and phenanthrene biliary metabolite concentrations were significantly different in *Trematomus bernacchii* captured at various sites in McMurdo Sound. The highest metabolite concentrations were in fish captured in WQB, where sediment ΣPAH levels exceeded 6000 ng · g^{-1} (Kennicutt *et al.* 1995). The lowest metabolite concentrations were measured in the bile of fish from Cinder Cones, a site considered to be relatively unimpacted. Unfortunately, no sediments were sampled at this site, but sediments collected across McMurdo Sound at Gneiss Point, Marble Point, Bernacchi Bay, and Bay of Sails contained low levels of PAH (<220 ng · g^{-1} Kennicutt *et al.* 1995). Biliary naphthalene and phenanthrene metabolite levels in fish from Cape Armitage were intermediate to those measured in fish from WQB and remote sites. Unlike fish from Arthur Harbor, *T. bernacchii* from WQB had significantly higher concentrations of ΣPAH in their tissues than fish from Cape Armitage and a remote site (Cinder Cones). Although ΣPAH levels in fish tissues are typically poor indicators of exposure, the significant site difference in PAH concentrations observed in fish from McMurdo Sound may be the result of chronic exposure to the high sediment PAH concentrations in WQB (Lenihan *et al.* 1990). There were no significant differences in BaP metabolite concentrations between WQB, Cape Armitage, and Cinder Cones. The lack of correlation between BaP metabolite data and ≥4-ring PAH concentrations in sediments is unknown. It is unlikely that the lack of site differences in BaP metabolite concentrations was the result of fish movement since significant differences in naphthalene and phenanthrene equivalent metabolites were observed in the same specimens. Possibly, a spectral interference at the BaP excitation/emission wavelength pairs for this species contributed to a lack of site differences. Unknown biogenic materials in the bile of Antarctic fish are suspected to have contributed to analytical interferences in previous studies (McDonald *et al.* 1992).

Fish were treated with DFA and BaP to establish the time-related induction of *in vivo* hepatic EROD activity and *in vitro* bioassay-derived TEQs for hepatic extracts of an Antarctic species. Fish were dosed with DFA because previous studies showed that DFA is the major contaminant in Arthur Harbor (Kennicutt *et al.* 1992a). Fish were also dosed with BaP because it is a model carcinogen and known to induce CYPIA in fish (Nishimoto *et al.* 1992; Varanasi *et al.* 1989). Maximum EROD activities were measured 144 h after the administration of BaP and DFA in fish; however, DFA-treated fish induced approximately 80% of the maximal EROD activity of BaP-treated fish. The relatively high induction response observed in fish dosed with DFA was unexpected since DFA is primarily composed of 2- and 3-ring PAH (Kennicutt *et al.* 1991) which are not considered to be CYPIA(I) inducers. Higher than expected CYPIAI induction was also observed in mammalian studies in which the induction potency of complex mixtures was evaluated. The potency of a complex mixture of PAH as inducers of hepatic EROD activity in B6C3F1 mice showed that the mixture was significantly greater than expected, based on the PAH composition (Chaloupka *et al.* 1993). The unexpected potency of DFA could be the result of unidentified potent P450IA inducers or unknown synergistic reactions (Chaloupka *et al.* 1993).

Rat hepatoma H4IIE cells were treated with hepatic extracts of fish dosed with DFA and BaP. The pattern of EROD induction and TEQs in H4IIE cells treated with hepatic extracts of DFA-dosed fish correlated with tissue concentrations of 3-ring PAH (correlation coefficient 0.65) and > 3-ring PAH (correlation coefficient 0.67), but not with ΣPAH (correlation coefficient 0.23) and 2-ring PAH (correlation coefficient 0.19). Generally, individual 3-ring and >3-ring PAH were below method detection limits; however, all non-zero values were summed to derive an approximate value for each ring category. A parallel relationship between hepatic BaP concentrations and *in vitro*-derived TEQs was observed using extracts from fish treated with BaP. The TEQs derived from the induction activity in H4IIE cells exposed to liver extracts of both DFA- and BaP-dosed fish were similar, again indicating the unexpected potency of the DFA mixture as an inducer. Extracts from only two fish captured near the Antarctic peninsula (off Palmer Station) induced EROD activity in rat hepatoma H4IIE cells, and the TEQs were approximately 10 to 100 × lower than those derived from dosed fish extracts.

In conclusion, data from this study provides evidence that fish captured near U.S. scientific stations are exposed to PAH, albeit low levels. Three biomarkers of PAH exposure were measured for this study and all were effective in evaluating contamination. *In vivo* hepatic EROD activities and *in vitro* EROD activities in rat hepatoma cells treated with liver extracts indicated that the induction activities were similar for both BaP- and DFA-treated fish and that the induction potency of DFA was higher than expected based on chemical composition. This suggests that it may be difficult to predict the CYPIA(I) induction potential of complex mixtures based on routine chemical analyses.

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