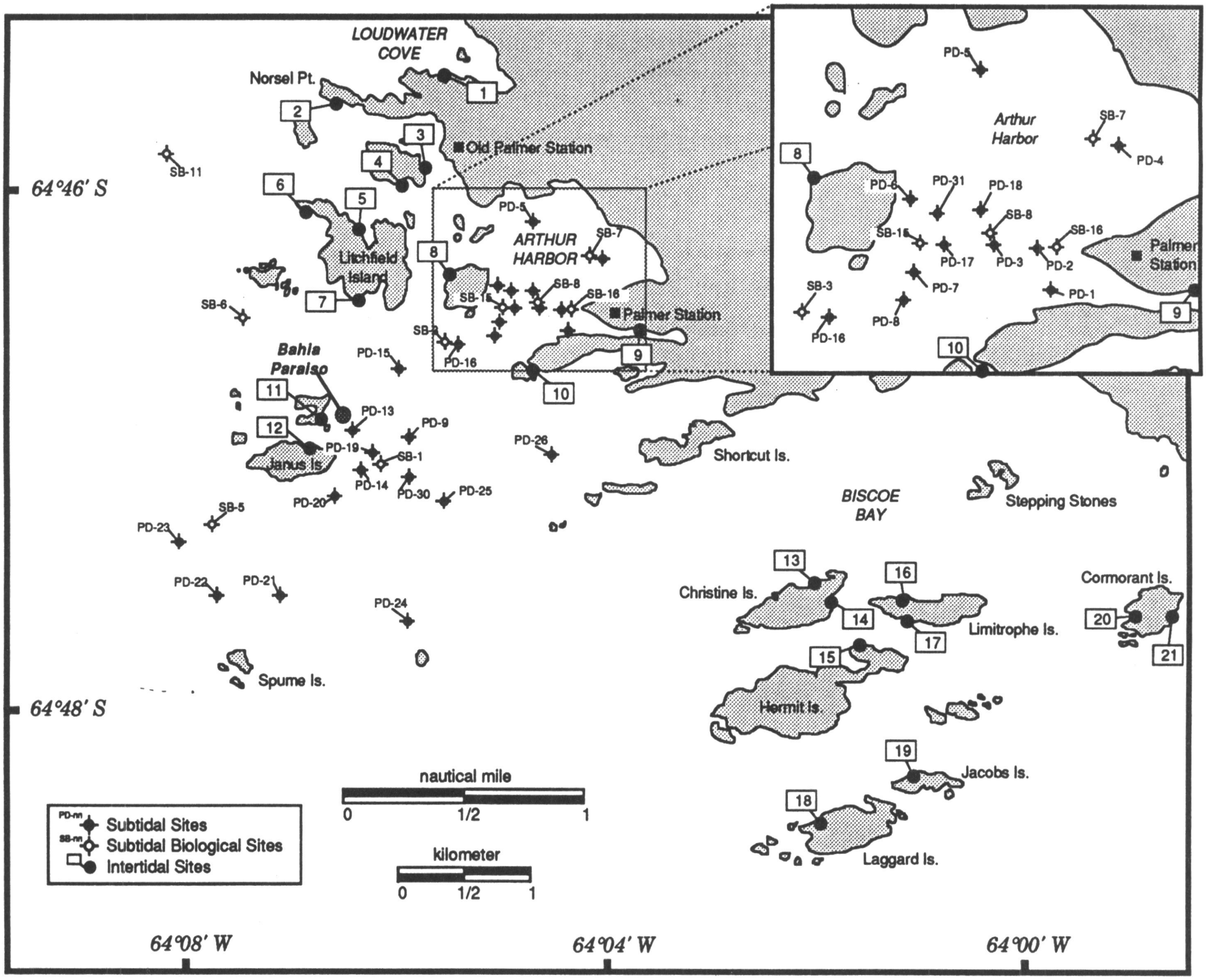

The location of intertidal and subtidal sampling locations for limpets and sediments in the vicinity of Arthur Harbor, Antarctica.

Polynuclear aromatic hydrocarbon exposure in antarctic fish

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Antarctica is considered to be one of the last remaining pristine environments. Consequently, much interest has been generated concerning the influence of humans on the antarctic environment. Recent studies have documented the presence of petroleum-related hydrocarbons in sediments and tissues sampled near Palmer Station and the *Bahia Paraiso* wreckage (Kennicutt et al. 1990, 1991a, b, and c this issue).

A study was undertaken to assess polynuclear aromatic hydrocarbon (PAH) exposure in fish. Fish enzymatically convert PAH into a variety of metabolites not detected by conventional techniques so measuring tissue concentrations in fish can underestimate PAH exposure. The metabolism of PAH is catalyzed by enzymes concentrated in the liver. Subsequently, metabolites can be excreted into bile for eventual elimination. The induction of mixed-function oxidase (MFO) enzyme activity has been widely used as a sensitive bioassay technique for the detection of organic contamination. The activity of ethoxyresorufin O-deethylase (EROD), a MFO enzyme, is commonly measured to suggest PAH

Table 1. Concentration of PAH metabolites in the bile of fish captured near Palmer Station, *Bahia Paraiso* wreck and remote sites

Site	Species	Naphthalene (ng g^{-1} wet wt.)	Phenanthrene (ng g^{-1} wet wt.)
Palmer Station	*Notothenia croiiceps neglecta*	51000 ± 9000 (n=6)	6000 ± 1500 (n=6)
Old Palmer Station	*Notothenia croiiceps neglecta*	21000 ± 6000 (n=2)	2500 ± 210 (n=2)
Bahia Paraiso	*Notothenia croiiceps neglecta*	69000 ± 61000 (n=16)	9000 ± 10000 (n=16)
Low Island	*Notothenia croiiceps neglecta*	38000 ± 14000 (n=9)	5000 ± 2000 (n=9)
Low Island	*Chaenocephalus aceratus*	19000 ± 13000 (n=8)	3000 ± 2000 (n=8)
Low Island	*Notothenia gibberfrons*	20000 ± 6000 (n=8)	2400 ± 930 (n=8)
Dallman Bay	*Notothenia gibberfrons*	36000 ± 17000 (n=6)	4000 ± 2200 (n=6)

Table 2. Biliary PAH metabolite concentrations in *Notothenia croiiceps neglecta* captured near the *Bahia Paraiso* wreck and Palmer Station

	Biliary PAH metabolites			
	Fluorescent aromatic hydrocarbons (ng g^{-1} wet wt.)		GC/MS (ng g^{-1} wet wt.)	
	Naph	Phen	290/335 nm Naph	260/350 nm Phen
Bahia Paraiso				
Fish 1	37000	2800	<LOD	<LOD
Fish 2	42000	4600	12	20
Fish 3	150000	18000	450	244
Fish 4	36000	3900	26	< LOD
Fish 5	35000	700	<LOD	<LOD
Fish 6	270000	37,000	1746	838
Palmer Station				
Fish 1	50000	8100	66	46
Fish 2	64000	8000	49	55
Fish 3	38000	6400	101	51

LOD = Limit of detection

exposure. Other techniques used to estimate PAH exposure in fish include measuring the concentration of metabolites in bile by high performance liquid chromatography (HPLC)/fluorescence detection and sequenced selected ion monitoring (SSIM) gas chromatography mass spectrometry (GC/MS) (Krahn et al. 1984, 1986, and 1992). The HPLC/fluorescence detection technique estimates metabolite concentrations by summing the integrated areas of all compounds eluting between specified elution times; whereas, GC/MS identifies and quantifies individual metabolites.

The bile of *Notothenia croiiceps neglecta* captured near Palmer Station and the *Bahia Paraiso* wreck and *Notothenia croiiceps neglecta, Notothenia gibberfrons,* and *Chaenocephalus aceratus* captured near remote sites (Low Island and Dallmann Bay) was screened by HPLC/fluorescence detection. The presence of metabolites fluorescing at naphthalene and phenanthrene wavelengths was detected in all samples (table 1). Higher than expected concentrations of metabolites were measured in the bile of fish sampled from remote sites. Therefore, the bile of selected fish was analyzed by GC/MS to confirm the presence of PAH metabolites. Individual PAH metabolites were identified by GC/MS in four fish captured near the wreck and in three captured near Palmer Station (table 2). The HPLC and GC/MS techniques both detected high concentrations of metabolites in two fish trapped near the *Bahia Paraiso* wreck. Additionally, the composition of metabolites is similar to that found in a *Notothenia gibberfrons* injected with diesel fuel. The three fish captured near Palmer Station contained low concentrations of individual metabolites indicating low-level exposure. GC/MS was unable to confirm

Table 3. Biliary PAH metabolite concentrations in fish captured near Low Island and in Dallman Bay

		Biliary PAH metabolites			
		Fluorescent aromatic hydrocarbons (ng g^{-1} wet wt.)		GC/MS (ng g^{-1} wet wt.)	
Site	Species	Naph	Phen	290/335 nm Naph	260/350 Phen
Dallman Bay	*Notothenia gibberfrons*	40000	4300	<LOD	<LOD
Low Island	*Notothenia croiiceps neglecta*	17000	2400	<LOD	<LOD
Low Island	*Notothenia gibberfrons*	22000	2200	<LOD	<LOD
Low Island	*Chaenocephalus aceratus*	24000	3200	<LOD	<LOD

LOD = Limit of detection

the presence of PAH metabolites in the bile of fish capture at remote sites (table 3). Furthermore, hepatic EROD activity was low (less than 50 pmol min^{-1} mg^{-1}) and did not appear to be induced in *Notothenia croiiceps neglecta* captured in Dallmann Bay. This suggests that these fish were not exposed to PAH. Apparently, compounds other than the analytes of interest fluoresce at the excitation/emission wavelengths selected to monitor naphthalene and phenanthrene metabolites.

Biliary metabolite concentrations indicate that fish captured near Palmer Station and the *Bahia Paraiso* wreck are exposed to PAH. Exposure near Palmer Station is most likely the result of chronic low-level input from boating/shipping and station activities (Kennicutt et al. this issue). Evidence for exposure in fish trapped near the wreck was variable. The variability in metabolite concentrations most likely reflects the heterogeneous distribution of contaminants in the sediments, episodic leakage from the wreck, and/or the mobility of the fish. Assessing PAH exposure in antarctic fish by screening bile using HPLC/fluorescence detection is useful but must be confirmed by other techniques to minimize the false positive indications of exposure caused by spectral interferences noted in some bile samples.

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Hydrocarbon contamination in Arthur Harbor, Anvers Island

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The presence of humans in remote areas can lead to the introduction of contaminants to otherwise pristine areas. It is often suggested that due to its remoteness, Antarctica should be one of the last remaining areas relatively free of the impact of human activities. However, human presence dates back to the early 1900s and has greatly increased in the last several decades (Platt and Mackie 1979; Clarke and Law 1981; Dayton 1972; Platt 1978, 1979; Lenihan et al. 1990). Localized contamination has been documented at several locations and in most instances is related to petroleum products (Cripps and Priddle 1991; Kennicutt et al. 1990; Lenihan et al. 1990; Risebrough et al. 1990). Petroleum, and its by-products, contain numerous compounds that have toxicological and carcinogenic effects. Due to the epidemiology of polynuclear aromatic hydrocarbons (PAH), they are commonly monitored in studies of anthropogenic pollution. Few studies have systematically documented the distributions, sources, and fate of contaminants in Antarctica (i.e., Kennicutt et al. 1990, 1991a, b, and c; Risebrough et al. 1990; Lenihan et al. 1990; Cripps 1990, 1991; Cripps and Priddle 1991).

The Antarctic Peninsula is a focus of human activity due to the concentration of bird and mammal populations, its mild climate, and its accessibility from South America. Many nations have located their scientific stations along the peninsula and the area attracts the majority of tourism activities in Antarctica. World attention was focused on pollution along the Antarctic Peninsula in 1989 when the Argentine supply ship the *Bahia Paraiso* ran aground in Arthur Harbor, Anvers Island (Kennicutt et al. 1990, 1991 a, b, and c). This incident highlighted the consequences of a catastrophic release of hydrocarbons (Kennicutt et al. this issue). Point source releases, such as spills, are dramatic events. However, a more insidious problem is long-term, sublethal exposure of marine ecosystems to contaminants due to everyday activities. We report the initial findings of a study of hydrocarbons in the organisms and sediments adjacent to Palmer Station, Anvers Island.

The major source of hydrocarbon contamination in subtidal samples near Palmer Station is diesel fuel spillage and leakage related to ship and boating activities (figure1). Combustion-derived PAH were present in subtidal sediments seaward of abandoned incineration sites at Palmer and Old Palmer Station (figures 1 and 2). Soil from various locations around Palmer Station was stained with weathered diesel fuel, lubricating oil, and hydraulic fluid. Subtidal sediments near Old Palmer Station were also contaminated with diesel fuel, apparently leaching from the shore. Diesel fuel was stored as a fuel cache and for heating fuel at Old Palmer Station. Spillage has contaminated soils and runoff transports a portion of this material to subtidal sediments. Soil and intertidal contamination was lower at Palmer Station than at Old Palmer Station even though Old Palmer Station has been inactive for years. This suggests that op-