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Changes in persistent contaminant concentration and CYP1A1 protein expression in biopsy samples from northern bottlenose whales, *Hyperoodon ampullatus*, following the onset of nearby oil and gas development

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Whale contaminants highlight concerns from oil and gas development near a marine protected area.

Abstract

A small population of endangered northern bottlenose whales (*Hyperoodon ampullatus*) inhabits "The Gully" a Marine Protected Area on the Scotian Shelf, eastern Canada. Amid concerns regarding nearby oil and gas development, we took 36 skin and blubber biopsy samples in 1996–1997 (prior to major development) and 2002–2003 (five years after development began), and three samples from a population in the Davis Strait, Labrador in 2003. These were analysed for cytochrome P4501A1 (CYP1A1) protein expression ($n = 36$), and for persistent contaminants ($n = 23$). CYP1A1 showed generally low expression in whales from The Gully, but higher levels during 2003, potentially coincident with recorded oil spills, and higher levels in Davis Strait whales. A range of PCB congeners and organochlorine compounds were detected, with concentrations similar to other North Atlantic odontocetes. Concentrations were higher in whales from The Gully than from the Davis Strait, with significant increases in 4,4′-DDE and *trans*-nonachlor in 2002–2003 relative to 1996–1997.

Keywords: Bottlenose whale; The Gully; PCB; DDT; Cytochrome P4501A1

1. Introduction

A small population of approximately 163 northern bottlenose whales (*Hyperoodon ampullatus*) has, as a large part of its habitat, the deeper waters within "The Gully", a submarine canyon off the coast of eastern Canada (Hooker et al., 2001b; Whitehead and Wimmer, 2005; Wimmer and Whitehead, 2004). Concern about the health of this population of cetaceans stems from the recent designation of The Gully as a Marine Protected Area, and the extraction of oil and gas from the surrounding shelf areas. For about three decades, there has been generally increasing interest in oil and gas exploration and production on the Scotian Shelf. In 1996, the Sable Offshore Energy Project began construction of five production rigs around Sable Island, with one of these lying only 30 km from The Gully (Fig. 1). These rigs began production operations in 1998.

Northern bottlenose whales are members of the beaked whale family, the Ziphiidae. This family is one of the least

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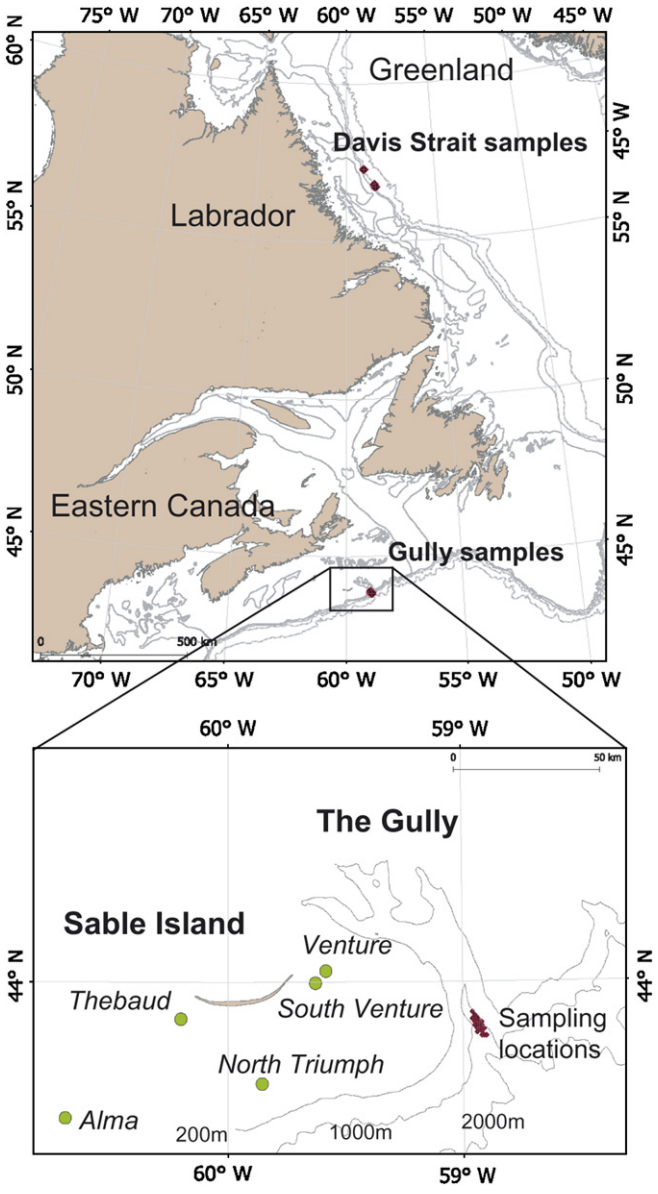

Fig. 1. Map showing location of The Gully and the Davis Strait populations of northern bottlenose whales. Expanded area shows The Gully region in the northwest Atlantic, with the locations of the recently developed oil and gas platforms shown (with names of the platforms).

known groups of marine mammals, largely due to their offshore habitat, long dive times, and shyness around vessels. Off the coast of eastern Canada, a population of northern bottlenose whales can be reliably found in The Gully, which appears to be an important foraging location (Hooker et al., 2001b). Unlike most other beaked whales, the northern bottlenose whale is relatively curious, and will approach stationary vessels (Mead, 1989). This has allowed research on the ecology and behaviour of these whales. A photo-identification study has shown that about one-third of the population using the waters off the Scotian Shelf reside in The Gully at any time (Gowans et al., 2000b; Whitehead and Wimmer, 2005). This population of whales is thought to be distinct from other populations in the North Atlantic, based on genetic differences (Dalebout et al., 2001, 2006) and size differences (Whitehead et al., 1997) observed between these whales and those from the nearest population in the Davis Strait.

The Gully has been recognized as an important habitat for several cetacean species (Hooker et al., 1999), but particularly for northern bottlenose whales. Concerns over the small size of the population and its susceptibility to anthropogenic impacts led to its listing by the Committee on the Status of Endangered Wildlife in Canada in 2002, and subsequent designation by the Canadian Government in 2006, as ''endangered''. After consideration and assessment over much of the last decade (Fenton et al., 2002), The Gully was designated as a Marine Protected Area in May 2004, in part to protect these whales.

There have been few previous reports on the levels and potential impacts of persistent contaminants in northern bottlenose whales. In this study, we compared the concentrations of PCBs and other organochlorine contaminants in skin and blubber biopsy samples collected from whales in The Gully before the start of oil and gas production (i.e. 1996–1997) and five years after production began (i.e. 2002–2003). We also compared contaminant concentrations in animals from the population in The Gully to concentrations in biopsy samples collected in 2003 from northern bottlenose whales living in the Davis Strait.

In addition, in order to evaluate exposure to biologically active contaminants, we analyzed biopsy samples for expression of cytochrome P4501A1 (CYP1A1) protein. Induction of CYP1A1 is a biomarker for exposure to contaminants that interact with the cellular aryl hydrocarbon (Ah) receptor (Stegeman et al., 1992) and induction of CYP1A1 is correlated to toxicity in rodents (Safe, 1986). Typical Ah-receptor ligands include the planar halogenated aromatic hydrocarbons such as the non- and mono-*ortho* substituted PCBs and certain polycyclic aromatic hydrocarbons. CYP1A1 is expressed in multiple cell types of cetacean skin (Angell et al., 2004) and this expression is induced by in vitro exposure to beta-napthoflavone, a prototypical Ah receptor ligand (Godard et al., 2004).

2. Methods

2.1. Biopsy collection

Field collections off eastern Canada took place approximately 300 km east of Halifax, Nova Scotia (Fig. 1), in The Gully submarine canyon (approximate position: 44°N, 59°W). Biopsy samples were collected from male and female bottlenose whales during August of 1996 and July–August of 1997 (i.e. pre-exploration), and during August of 2002, and August–September of 2003. Field collections in the Davis Strait took place approximately 200 km east of Nain, Labrador (approximate position: 57°N, 58°W), where biopsies were collected from male and female whales in August of 2003 (Fig. 1).

Biopsy sampling was conducted from either a 12-m auxiliary sailing vessel or from a 5-m rigid-hulled inflatable tender. These vessels were operated under power at speeds of 1–4 knots during collection of biopsy samples. Biopsy darts (2.5-cm long, 0.6-cm diameter) were fired from a 150 lb crossbow (Barnett Wildcat XL) or from a Paxarms air-rifle at ranges of 5–15 m, as described by Hooker et al. (2001a). All samples were taken from the flank near the dorsal

fin. We attempted to sample adult whales (based on field observations of animal size) and avoided sampling of calves.

Each biopsy sample was subdivided for the different types of analysis within an hour of collection to allow matched subsamples from each animal. Subsamples of skin were stored in DMSO for genetic analysis of sex (Gowans et al., 2000a). A biopsy subsample consisting of the skin–blubber interface was stored in neutral buffered formalin for analysis of the expression of CYP1A1. A subsample of blubber from each of the biopsies was stored temporarily in hexane-washed foil in liquid nitrogen, then kept frozen in hexane-washed glassware with teflon-lined lids at −20 °C when freezer facilities became available, for analysis of contaminant concentrations.

2.2. Biopsy analysis

The sex of the biopsied whales was determined by genetic analysis of the skin using the method described by Gowans et al. (2000a). Expression of CYP1A1 protein was determined using an immunohistochemical method described by Angell et al. (2004). Briefly, the samples (approximately 0.5 g) were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 5 μm. Serial sections were deparaffinated, hydrated and stained immunohistochemically using a peroxidase/antiperoxidase detection system (Signet Laboratories, Dedham, MA, USA) with the monoclonal antibody (MAb) 1-12-3 against scup (*Stenotomus chrysops*) CYP1A as the primary antibody and non-specific MAb MOPC or UPC-10 (Sigma–Aldrich, St. Louis, MO, USA) as the control antibody. Mab 1-12-3 recognizes an epitope that occurs in CYP1A1 but not CYP1A2 in mammals (Drahushuk et al., 1998). Amino-9-ethylcarbazole (Signet Laboratories) was used as the chromogenic substrate for visualization of CYP1A1. Sections were counterstained with Mayer's hematoxylin (Sigma–Aldrich) for visualization of nuclei. Stained sections were evaluated under light microscopy for stain occurrence (scale of 0–3) and stain intensity (scale of 0–5) in each cell type. CYP1A1 expression was calculated as the product of stain occurrence and intensity to provide a semi-quantitative index (scale 0–15).

Blubber from biopsy samples was prepared for analysis of contaminants as described by Metcalfe and Metcalfe (1997). Briefly, blubber samples of <1 g were mixed and ground with sodium sulfate and packed into a glass column for extraction into 50:50 dichloromethane/hexane using a cold-column extraction procedure. The lipids were removed from extracts by gel permeation chromatography (GPC) and the lipid fraction was collected for gravimetric analysis of the lipid content of the sample. The GPC fraction containing contaminants was subfractionated by silica-gel column chromatography to yield Fraction A that contained primarily PCB congeners and some DDE, and Fraction B that contained the rest of the DDE and the majority of the organochlorine analytes.

The samples were analyzed by gas chromatography with an electron capture detector (GC-ECD), as described previously (Metcalfe and Metcalfe, 1997). All samples were analyzed for 18 major PCB congeners (denoted as major PCB congeners) with IUPAC numbers 44, 49, 52, 87, 99, 101, 105, 110, 118, 138, 149, 153, 156, 170, 180, 194, 195, 209. Samples collected in 2002–2003 were analyzed for additional minor PCB congeners with IUPAC numbers 17, 18, 28, 31, 33, 47, 66, 70, 74, 95, 128, 132, 151, 187, 191, 199, 201, 205, 206. The samples were also analyzed for a range of organochlorine compounds, including 2,4′- and 4,4′-isomers of DDT (dichlorodiphenyltrichloroethane), and its metabolites DDE (dichlorodiphenylchloroethylene) and DDD (dichlorodiphenyldichloroethane), α-HCH (hexachlorocyclohexane), β-HCH, δ-HCH, and γ-HCH, *trans*- and *cis*-chlordane, *trans*- and *cis*-nonachlor, endosulfan, endosulfan II and endosulfan sulfate, heptachlor and heptachlor epoxide, aldrin, dieldrin, endrin, endrin ketone and endrin aldehyde, methoxychlor, mirex, hexachlorobenzene (HCB) and octachlorostyrene. The Limits of Detection (LOD) were between 0.04 and 0.25 ng/mL for individual PCB congeners, between 0.05 and 0.1 ng/mL for DDT and metabolites and between 0.01 and 0.5 ng/mL for all other organochlorine analytes. Procedural blanks and a National Institute for Standards and Technology cod liver oil reference material (SRM 1588) were analyzed for quality control/quality assurance purposes.

The total PCB concentration was calculated as the sum of the "major PCB congeners" and the total DDT concentration was calculated as the sum of 4,4′- and 2,4′-isomers of DDT, DDE and DDD. The total HCH concentration was calculated as the sum of the four HCH isomers. Total chlordane was calculated as the sum of *trans*- and *cis*-chlordane and *trans*- and *cis*-nonachlor. Total endosulfan was calculated as the sum of endosulfan and its two metabolites. All analyte concentrations were lipid normalized (i.e. ng/g lipid).

2.3. Statistical analysis

Means and standard deviations about the mean were calculated for lipid-normalized concentrations of contaminants, and these data are presented for reference in all tables and figures. Since data were in some cases slightly skewed, we used log-transformed data for all statistical analysis. Thus, in cases where analytes were not detected, we used the detection limit as a default value and surrogate concentrations equivalent to the specific LOD for each congener were substituted prior to log-transformation. Differences in the concentrations of total analytes (total PCBs, total DDTs, etc.) in sample groups were tested using a MANOVA with the log-transformed data for the lipid-normalized concentrations of selected contaminants. Differences between CYP1A1 expression in endothelial and smooth muscle cells between years and locations were tested using a MANOVA. Statistical analyses were conducted with SPSS 11.5.

3. Results

Biopsy samples were collected from 39 bottlenose whales (18 males, 21 females) during the study. However, since the biopsies did not always contain sufficient material for both types of analyses, CYP1A1 analysis was conducted with 36 samples, and contaminant analysis was conducted with 23 samples (Table 1). Although we attempted to photograph all animals that were sampled, photographs were not always of sufficient quality or animals were not marked well enough for identification. Approximately 50% of the animals that were biopsied were identified from photographs. From photographic identification, it was determined that one animal (a mature male, ID 480) was sampled in both the 1996–1997 and 2002–2003 time periods (Table 1).

Since expression of CYP1A1 in tissues is thought to reflect recent exposure to contaminants that act through the Ah-receptor (Fossi et al., 1994), the CYP1A1 results are presented for each year of collection (Fig. 2). In general, scores for CYP1A1 activity in biopsies from the bottlenose whales were low, with the majority of samples (i.e. 30 of 36 endothelial and 27 of 36 smooth muscle) scoring zero (Table 1). Some limited numbers of animals expressed CYP1A1 in endothelial and vascular smooth muscle cells. Samples collected from The Gully in 2003 showed significantly higher expression for CYP1A1 than samples collected in 1996, 1997 or 2002 (MANOVA $F = 9.249$, $p < 0.001$). Surprisingly, although only three samples were analysed from whales in the Davis Strait, Labrador, these showed relatively high scores for CYP1A1 expression (endothelial 3.5 ± 0.87 and smooth muscle 6.33 ± 2.52, $n = 3$, Fig. 2, Table 1). These scores were significantly greater than those for whales from The Gully (MANOVA, $F = 34.110$, $p < 0.001$). There was no significant difference between expressions of CYP1A1 in samples based on sex of whale (MANOVA using location and year as covariates, $F = 0.705$).

A range of PCB congeners and organochlorine contaminants were detected in the blubber biopsies collected from

Table 1
Location, dates and times of collection, sex, ID (where determined) and analytical history for biopsy samples collected from northern bottlenose whales for analysis of concentrations of persistent contaminants and activity of CYP1A1 enzyme

Location	Date	Time	Sex	ID	Contaminants	CYP1A1 Endo/Sm muscle
The Gully	27 Aug 96	10:28	m	–	–	0/0
	27 Aug 96	11:05	m	143	–	0/0
	16 July 97	12:00	f	1289	x	0/0
	16 July 97	15:36	f	–	x	0/0
	16 July 97	17:20	f	–	x	0/0
	16 July 97	19:32	f	54	–	0/0
	12 Aug 97	14:48	f	961	–	
	12 Aug 97	16:07	f	1000	x	0/0
	12 Aug 97	18:53	f	1313	–	0/0
	12 Aug 97	19:18	f	–	–	0/0
	13 Aug 97	14:57	f	1318	–	0/0
	13 Aug 97	16:33	f	1315	–	0/0
	13 Aug 97	16:44	f	619	–	0/0
	14 Aug 97	07:50	f	–	x	0/0
	14 Aug 97	10:00	m	–	x	0/0
	14 Aug 97	10:07	m	480[a]	x	0/0
	16 Aug 97	08:59	m	1039	x	0/0
	16 Aug 97	10:00	m	–	x	0/0
	16 Aug 97	12:22	f	1336	–	3/0
	16 Aug 97	13:16	m	–	x	0/0
	08 Aug 02	14:31	f	677	x	0/0
	08 Aug 02	14:42	m	76	x	0/0
	08 Aug 02	16:57	m	3	x	0/0
	14 Aug 02	09:18	f	–	x	0/0
	14 Aug 02	09:27	m	–	–	0/0
	14 Aug 02	11:38	f	–	x	0/0
	14 Aug 02	12:17	m	–	–	0/0
	14 Aug 02	15:08	f	251	x	0/0
	14 Aug 02	15:19	f	102	–	–
	29 Aug 03	07:08	m	–	–	0/1.5
	29 Aug 03	07:18	m	–	x	2/5
	29 Aug 03	12:01	m	–	x	0/2.5
	29 Aug 03	12:50	m	824	x	4.5/2.5
	29 Aug 03	19:38	m	480[a]	x	–
	25 Sept 03	14:09	f	–	–	0/2
	25 Sept 03	19:04	f	–	–	0/1.5
Davis Strait	14 Aug 03	11:53	f	–	x	3/6
	16 Aug 03	09:59	m	2209	x	3/9
	16 Aug 03	18:16	m	–	x	4.5/4

Scores for expression of cytochrome P4501A1 (CYP1A1) in the vascular endothelial layer (Endo) and smooth muscle layer (Sm muscle) is shown. The CYP1A1 scale ranges from 0 to 15. m = male, f = female.
[a] The same male was sampled in both 1996 and 2003.

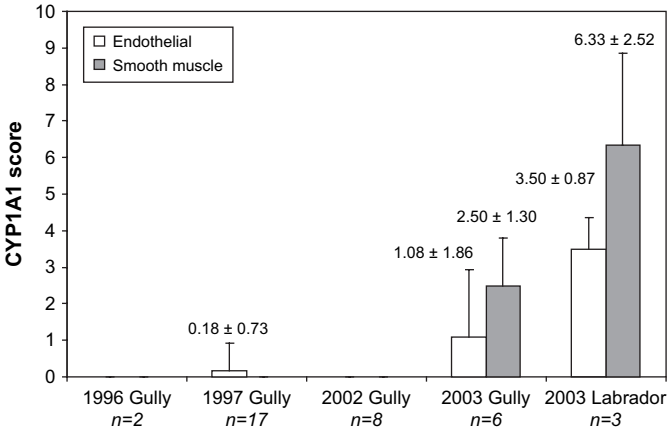

Fig. 2. Scores for expression of cytochrome P4501A1 (CYP1A1) in the vascular endothelial layer and smooth muscle layer of biopsy samples from northern bottlenose whales for each year and location sampled. Mean (±S.D.) are shown.

northern bottlenose whales (Table 2). The classes of compounds present in the highest concentrations were (in order) DDT and its metabolites, PCBs, chlordanes, HCHs, and dieldrin. There was considerable variation in the concentrations of compounds in different individuals. There were significant differences between contaminant concentrations (total PCBs, HCHs, DDTs, chlordanes and endosulfan) for male and female whales (MANOVA using location and sample year as covariates, $F = 7.438$, $p = 0.001$). The mean concentrations in females were lower than those in males (Table 2).

For most contaminants, the concentrations observed in whales sampled in the Davis Strait were much lower than the concentrations in whales from The Gully (Fig. 3, Table 2, MANOVA using sex as a covariate, $F = 6.162$, $p = 0.002$).

Among whales from The Gully region, there was a significant difference between contaminant concentrations (total PCBs, HCHs, DDTs, chlordanes and endosulfan) in samples collected in 1996–1997 and those in 2002–2003 (MANOVA using sex as a covariate, $F = 11.7$, $p < 0.001$). Of these contaminants, total PCBs showed no significant differences, DDTs and chlordanes showed significant increases, while HCHs and endosulfans showed significant decreases (Table 2, Fig. 3). Total PCB concentrations in whales from The Gully were 7273 and 3791 ng/g lipid for males and females, respectively, sampled in 1996–1997, and 6841 and 4061 ng/g lipid for males and females, respectively, sampled in 2002–2003. Concentrations of total DDT in whales from The Gully increased by 50% from 14,658 and 4143 ng/g lipid for males and females, respectively, sampled in 1996–1997, to 21,571 and 7601 ng/g lipid for males and females, respectively, sampled in 2002–2003. Concentrations of total chlordanes in whales from The Gully increased by 100% from 909 and 475 ng/g lipid for males and females, respectively, sampled in 1996–1997, to 1809 and 1189 ng/g lipid for males and females, respectively, sampled in 2002–2003. Closer investigation of the individual components of these classes of compounds indicates that the differences in contaminant concentrations can be primarily attributed to 4,4′-DDE and *trans*-nonachlor (Figs 4 and 5).

The same pattern of increase in contaminant concentrations was observed for biopsies from a single male animal that was re-sampled in The Gully area. As shown in Table 2, in this animal, there was little change in total PCBs, and a decrease in total HCH, while total chlordanes increased from 638 to 797 ng/g lipid. However, there was a threefold increase in total DDT concentration, with a large increase in the concentration of 4,4′-DDE. A large increase was also observed in dieldrin concentration, from 500 to 3432 ng/g lipid (Table 2).

Table 2
Mean and standard deviations about the mean of concentrations (ng/g lipid) of PCBs and organochlorine compounds in blubber biopsies of northern bottlenose whales from The Gully and the Davis Strait, Labrador

Analyte	Gully 1996−1997				Gully 2002−2003				Davis Strait 2003			# 480, 1996	# 480, 2003
	Males mean $n=5$	S.D.	Females mean $n=5$	S.D.	Males mean $n=6$	S.D.	Females mean $n=4$	S.D.	Males mean $n=2$	S.D.	Female $n=1$	Male $n=1$	Male $n=1$
PCB congeners													
18	na	na	na	na	6.5	10.1	5.9	5.8	4.9	1.5	nd	na	nd
17	na	na	na	na	0.0	0.0	0.5	0.9	1.3	1.8	nd	na	nd
31	na	na	na	na	7.8	15.5	13.6	10.9	nd	−	nd	na	nd
28	na	na	na	na	0.1	0.3	1.9	3.7	9.0	0.5	3.5	na	nd
33	na	na	na	na	nd	−	1.4	2.8	nd	−	nd	na	nd
52	534.2	170.7	258.1	213.5	401.7	278.2	199.6	91.9	161.8	9.7	20.6	288.9	286.5
49	145.4	81.9	85.6	55.2	110.4	72.7	56.8	28.7	42.0	0.1	6.5	50.5	10.5
47	127.6	47.6	144.5	68.0	na	na	na	na	na	na	na	75.4	na
44	109.0	58.9	35.6	34.7	70.0	43.1	33.8	27.6	40.5	5.5	nd	62.6	38.8
66	16.7	37.2	52.9	38.7	na	na	na	na	na	na	na	nd	na
74	na	na	na	na	93.5	109.9	12.3	24.5	66.1	25.7	3.2	na	58.5
70	na	na	na	na	11.0	17.5	34.1	31.9	18.1	6.0	3.5	na	nd
95	na	na	na	na	346.5	272.0	120.1	55.8	160.2	17.6	25.0	na	272.6
101	826.2	219.1	403.3	214.3	449.6	284.0	286.7	133.3	198.1	15.5	26.5	539.9	263.4
99	365.5	74.8	197.0	121.7	662.5	348.0	647.5	327.4	906.8	254.5	28.0	257.1	450.0
87	270.2	70.5	122.4	68.4	160.5	98.6	99.0	48.6	41.5	2.7	6.9	169.5	91.8
110	133.0	57.0	121.2	90.6	18.3	26.4	108.0	128.5	47.3	43.4	17.4	94.9	7.1
151	na	na	na	na	1584.1	2138.7	210.5	351.8	2399.2	65.8	376.6	na	866.9
149	532.3	98.9	258.5	123.0	636.2	412.4	407.5	238.4	229.5	42.1	28.6	376.0	466.9
118	722.2	161.3	389.9	211.4	651.3	428.7	403.7	177.9	443.5	152.1	36.8	475.1	450.6
153	1209.8	241.6	680.1	234.2	1515.4	842.2	768.3	361.5	666.5	28.0	140.3	861.9	1394.0
132	na	na	na	na	175.7	138.6	81.2	45.5	81.6	10.0	7.7	na	149.8
105	134.3	28.6	96.3	40.1	97.6	85.8	49.7	42.7	129.4	57.4	13.1	99.6	78.2
138	1284.4	226.5	671.2	241.9	1387.9	863.1	608.3	280.2	425.4	133.3	84.6	941.6	1424.6
187	na	na	na	na	392.1	187.9	234.7	78.3	166.5	80.4	53.5	na	340.1
128	na	na	na	na	158.4	64.3	191.0	206.0	52.4	22.0	8.7	na	106.8
156	73.6	22.1	35.3	18.4	55.2	41.1	31.4	11.2	12.7	3.1	5.4	44.7	47.5
180	574.3	169.5	267.9	33.4	443.4	213.9	244.7	66.0	149.0	33.8	58.2	353.5	386.3
191	na	na	na	na	1.2	2.2	1.3	0.9	nd	−	nd	na	nd
170	236.8	68.5	112.7	30.3	133.3	60.0	71.7	10.9	49.9	20.7	20.2	148.4	113.8
199	198.9	81.1	92.5	26.1	na	na	na	na	na	na	na	105.7	na
201	na	na	na	na	93.4	51.4	49.3	15.7	31.2	15.1	32.9	na	78.3
195	22.9	11.4	13.4	11.6	5.4	10.1	5.2	4.0	1.7	2.4	5.8	7.6	0.6
194	87.9	39.6	37.6	18.1	39.5	33.6	24.6	15.8	10.8	7.9	24.0	45.9	33.3
205	na	na	na	na	0.7	1.8	1.1	0.9	0.7	1.0	nd	na	nd
206	na	na	na	na	13.0	22.2	9.0	8.5	4.9	3.4	15.1	na	11.0
209	11.1	15.3	4.9	10.9	3.3	8.1	3.0	3.3	1.4	1.9	5.8	nd	nd
Total PCBs[a]	7273.1	1704.1	3791.1	1665.9	6841.4	3868.6	4061.9	1682.0	3557.8	268.0	528.6	4817.5	5543.9

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Table 2 (*continued*)

Analyte	Gully 1996–1997				Gully 2002–2003				Davis Strait 2003			# 480, 1996	# 480, 2003
	Males mean $n=5$	S.D.	Females mean $n=5$	S.D.	Males mean $n=6$	S.D.	Females mean $n=4$	S.D.	Males mean $n=2$	S.D.	Female $n=1$	Male $n=1$	Male $n=1$
Organochlorines													
α-HCH	47.4	34.2	32.0	13.2	12.4	5.3	16.0	8.7	9.1	1.0	9.9	36.9	6.1
β-HCH	nd	–	nd	–	6.3	7.4	11.1	12.4	nd	–	nd	na	nd
γ-HCH	65.5	38.6	18.5	14.7	4.5	3.5	10.9	4.6	0.5	0.8	1.2	57.9	2.5
δ-HCH	96.1	109.3	6.6	14.8	0.6	0.6	1.2	2.4	0.0	0.0	0.0	73.8	0.7
Total HCHs	209.0	169.8	57.1	23.5	23.8	14.3	39.2	3.9	9.7	1.8	11.0	168.6	9.2
trans-Chlordane	271.1	232.3	25.9	20.0	13.5	12.5	15.4	7.1	nd	–	30.0	242.9	nd
cis-Chlordane	191.0	105.9	48.1	13.0	75.9	36.8	46.9	16.6	1.6	0.3	24.4	118.6	92.9
trans-Nonachlor	229.2	103.6	254.9	131.5	1420.3	821.9	949.9	277.6	259.7	8.6	203.6	106.8	63.8
cis-Nonachlor	217.6	35.3	145.6	63.4	299.5	197.8	176.3	38.6	nd	–	50.6	170.1	641.1
Total chlordane	908.9	293.2	474.5	208.5	1809.2	704.6	1188.6	217.1	261.3	8.3	308.7	638.3	797.8
2,4′-DDE	45.9	33.1	31.9	22.5	120.1	93.9	51.6	35.6	0.0	0.0	2.4	0.0	261.9
4,4′-DDE	8218.2	5846.8	1887.9	506.9	14441.7	10398.5	4351.2	1946.4	3080.6	938.4	600.7	4815.5	9271.8
2,4′-DDD	184.1	44.2	75.5	30.3	96.3	88.2	54.7	17.4	3.6	5.1	nd	117.3	143.0
4,4′-DDD	2312.4	748.2	796.1	468.6	1867.8	1189.7	1128.9	394.0	269.5	14.9	136.6	1197.5	1772.2
2,4′-DDT	237.9	111.1	177.0	107.8	916.4	760.9	357.1	253.8	43.5	16.4	75.1	87.8	1075.5
4,4′-DDT	3677.3	2470.0	1174.5	588.3	4129.3	3644.2	1658.4	926.6	179.4	135.1	261.5	1146.8	10403.5
Total DDT	14658.3	6693.9	4143.0	1590.7	21571.5	10829.0	7601.8	1485.4	3576.6	1037.0	1076.3	7277.1	22927.9
Endosulfan	158.6	158.2	9.2	14.0	0.9	2.3	15.8	28.7	22.5	31.8	nd	127.1	nd
Endosulfan II	171.1	109.8	44.4	20.0	73.2	78.3	0.0	0.0	17.8	8.4	45.7	126.8	213.4
Endosulfan sulfate	281.8	297.7	41.7	20.2	28.3	25.8	27.7	18.7	0.0	0.0	9.9	198.1	23.2
Total endosulfan	611.6	562.6	95.2	17.8	102.4	67.5	43.5	10.2	40.3	23.4	55.6	452.0	236.5
Heptachlor	6.1	9.8	nd	–	1.6	3.7	1.1	1.3	nd	–	nd	nd	9.1
Heptachlor epoxide	na	na	na	na	1045.5	1197.6	228.2	182.1	117.1	1.1	49.9	na	3001.0
Dieldrin	672.5	209.7	393.0	319.7	1162.8	1196.2	626.8	144.0	192.1	22.7	106.9	501.0	3432.0
Aldrin	nd	–	nd	–	nd	–	1.8	3.5	nd	–	nd	nd	nd
Endrin	234.5	153.0	53.1	51.9	182.7	347.4	266.4	243.5	nd	–	nd	194.2	nd
Endrin aldehyde	na	na	na	na	6.1	10.1	11.1	7.6	nd	–	nd	na	nd
Endrin ketone	84.0	88.0	2.7	6.0	41.1	25.1	61.4	0.1	nd	–	4.0	102.9	78.2
Methoxychlor	180.5	202.8	6.5	13.0	0.0	0.0	124.9	0.1	nd	–	nd	125.0	0.0
Mirex	30.0	13.0	19.7	5.6	35.6	15.2	13.9	0.1	19.7	9.9	13.7	29.6	34.3
HCB	236.4	103.7	254.2	223.7	325.2	413.3	46.6	93.2	559.8	16.5	35.8	227.9	269.3
Octachlorostyrene	49.0	24.5	13.1	11.7	9.1	14.9	12.1	15.4	nd	–	nd	68.4	nd

nd = not detected, na = not analyzed.

[a] Total PCBs calculated only for the major PCB congeners in bold, as these were analyzed in samples collected in both 1996–1997 and 2002–2003.

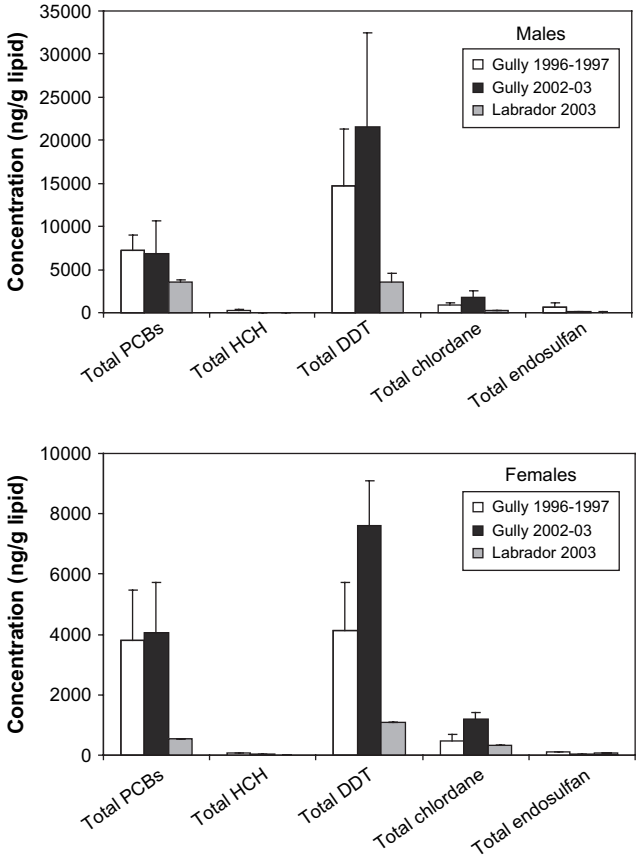

Fig. 3. Mean concentrations (±S.D.) in ng/g lipid of total PCBs and classes of organochlorine compounds in blubber biopsies taken from bottlenose whales in The Gully in 1996–1997, versus those in 2002–2003, and those taken from whales in the Davis Strait in 2003. Separate plots are shown for male and female whales.

4. Discussion

This study uses biopsy sampling to assess contaminant levels in blubber and expression of cytochrome P450 enzymes in the skin of odontocetes. One concern of such studies is that biopsies only sample the superficial blubber layers, and that there may be vertical stratification of lipid types within the blubber mantle (Koopman et al., 1996). However, in blubber removed from baleen whales, lipid-normalized contaminant levels showed no significant differences according to body site or blubber strata (Gauthier et al., 1997). On the other hand, while concentrations of PCBs and organochlorine pesticides in superficial blubber biopsies collected from free-ranging St. Lawrence beluga whales (*Delphinapterus leucas*) were consistent with the concentrations in full depth blubber samples removed from stranded belugas, there were differences between the biopsies and full depth samples in the relative proportions of highly chlorinated PCB congeners, HCB, DDT isomers and mirex (Hobbs et al., 2003). Stratification of dietary fatty acids in the blubber layer of northern bottlenose whales is much less than that observed among other odontocete species, such as harbour porpoises (*Phocoena phocoena*) (Hooker et al., 2001b), so the outer blubber layer may be representative of all blubber strata. Furthermore, although outer blubber may be less physiologically dynamic and more structural in function, this may in fact serve as a better measure of integrated long-term persistent contaminant exposure. Therefore, it was assumed that the biopsy method was an appropriate sampling technique for assessing the concentrations of PCBs and organochlorine contaminants in the blubber of bottlenose whales.

4.1. Bottlenose whale contaminant levels

Contaminant concentrations have been previously reported for northern bottlenose whales in two studies. A juvenile male stranded in the North Sea in 1976 contained 37,100 ng/g total PCBs and 14,200 ng/g total DDT in blubber on a wet weight basis (Harms et al., 1978). Oil from a northern bottlenose whale caught in the Canadian fishery in 1962 (which would have been from The Gully region, according to Reeves et al., 1993) contained appreciable amounts of contaminant residues, including total DDT at 11,600 ng/g of oil, and PCBs at 1000 ng/g of oil (Addison et al., 1972). The DDT in this sample was composed of approximately equal parts of 4,4′-DDE and 4,4′-DDD, but 4,4′-DDT was not detected. Some of these differences in contaminant levels between the present study and these earlier reports for bottlenose whales are probably due to recent advances in analytical techniques, such as the congener-specific analysis of PCBs. Unfortunately, this makes any inference regarding historical changes in contaminant levels unreliable.

The concentrations of contaminants found in the blubber of northern bottlenose whales are generally consistent with concentrations reported for other large odontocetes in the North Atlantic (Table 3). It is generally accepted that maternal transfer of hydrophobic contaminants in marine mammals reduces the concentrations of these compounds in females relative to males. The large variability seen in the concentrations of contaminants in whales is probably due to differences in sex, age and size of the animals that were biopsied (Jepson et al., 2005; Muir et al., 1996; Ross et al., 2000; Stern et al., 1994; Subramanian et al., 1988; Tuerk et al., 2005; Weisbrod et al., 2000). The DDT/PCB ratio in whales has been suggested to correlate to distance of ecosystem location from the mainland (Aguilar, 1987). Our results demonstrated high DDT/PCB ratios for bottlenose whales, consistent with other beaked whales and pilot whales (*Globicephala melas*), but contrary to Atlantic sperm whales (*Physeter macrocephalus*), arctic narwhal (*Monodon monoceros*) and beluga (Table 3). However, Stern et al. (1994) observed that these ratios in arctic belugas varied with age, especially in females due to differential loss of DDT and PCBs.

The concentrations of total DDT and total PCBs in the blubber of bottlenose whales were lower than the concentrations reported in highly contaminated odontocete species (e.g., total PCB concentrations of >250,000 ng/g lipid for killer whales in British Columbia, Canada, Ross et al., 2000; and total PCB concentrations of >60,000 ng/g lipid, and 4,4′-DDE concentration >16,000 ng/g lipid for belugas in the St. Lawrence estuary, Canada, Muir et al., 1996). Total

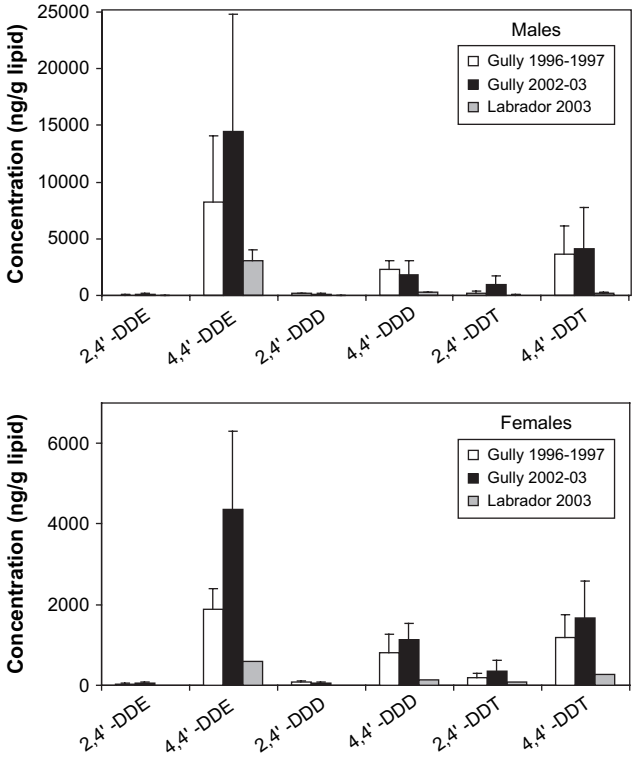

Fig. 4. Mean concentrations (±S.D.) of 2,4′- and 4,4′-isomers of DDE, DDD and DDT in ng/g lipid from blubber biopsies collected from male and female bottlenose whales in The Gully in 1996–1997 and in 2002–2003, and in the Davis Strait in 2003.

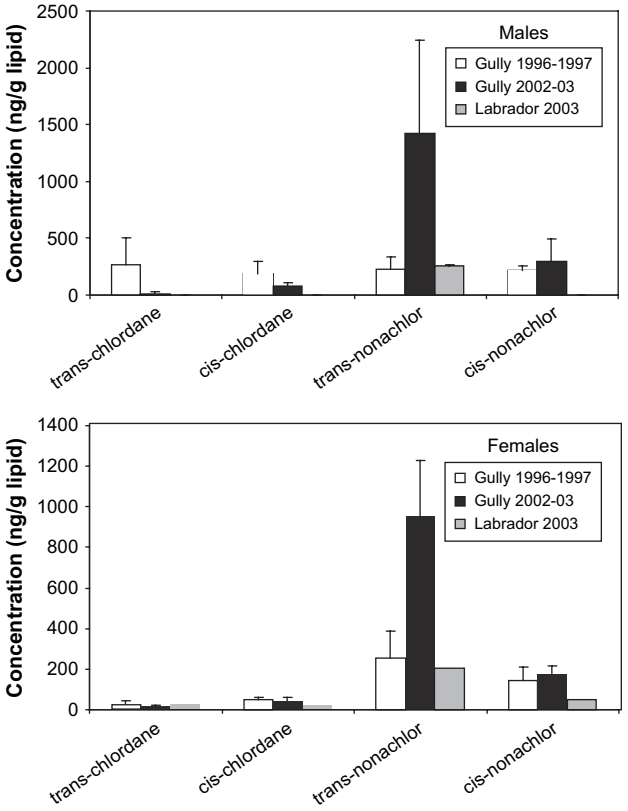

Fig. 5. Mean concentrations (±S.D.) of *cis*- and *trans*-isomers of chlordane and nonachlor in ng/g lipid from blubber biopsies collected from male and female bottlenose whales in The Gully in 1996–1997 and in 2002–2003, and in the Davis Strait in 2003.

blubber PCB levels >17,000 ng/g lipid have been associated with an elevated prevalence of infectious diseases in harbor porpoises in the UK (Jepson et al., 2005). Schwacke et al. (2002) estimated an excess risk of reproductive failure in bottlenose dolphins (*Tursiops truncatus*) from the coast of the southeast USA with total blubber PCB concentrations of between 4200 (adult female) and 91,200 ng/g lipid (adult male). Hall et al. (2006) used population models to predict that high concentrations of PCBs in bottlenose dolphins in Florida, USA would depress the growth of the population. However, the concentrations of persistent contaminants, and in particular PCBs, in the bottlenose whales from The Gully region were lower magnitude than these deleterious concentrations.

The CYP1A1 analyses showed low levels of expression of this cytochrome P450 enzyme. It is thought that CYP1A1 induction only persists for the duration of exposure (Fossi et al., 1994). Compounds that can induce CYP1A1 include coplanar PCB congeners, halogenated dioxins and dibenzofurans and polynuclear aromatic hydrocarbons (PAHs) (Safe, 2002). Of the contaminants we sampled in the blubber it is only the few mono-*ortho* and non-*ortho* PCBs that would be capable of inducing CYP1A1. The other PCBs and organochlorine contaminants would not induce CYP1A1. Our analysis confirms that contaminants found in the blubber did not correlate with induction of the cytochrome P4501A1 class of enzymes in the skin. This indicates that the planar PCBs detected in the blubber layer were not being actively mobilized and circulated to the skin during or near sampling of these animals. Considering that lower concentrations were found in the animals expressing the highest levels of CYP1A1 (Davis Strait samples) and there was no change in any measured contaminant that also interacts with the Ah receptor in the Gully animals over time, the causative agent inducing CYP1A1 was not measured in this study and remains unknown. No attempt was made to evaluate exposure of the bottlenose whales to PAHs, since these compounds are rapidly metabolized and do not persist in the tissues of vertebrates.

4.2. Changes detected between 1996–1997 and 2002–2003 for samples from The Gully

There was a significant increase observed in the concentrations of some contaminants (e.g. 4,4′-DDE, *trans*-nonachlor) in whales from The Gully over the five-year time span from 1996–1997 to 2002–2003. There could be many reasons for this observation, including metabolic changes in contaminant proportions, changes in feeding patterns or in the geographic range of the animals. Over time DDT is metabolized into several forms, of which DDE is a derivative that is difficult to degrade or excrete and is thus accumulated (Aguilar, 1984). However, since we have observed increases in total DDTs in addition to some increase in DDE/total (DDT) proportion, it is unlikely that the increased 4,4′-DDE observed is simply

Table 3
Concentrations of total PCB (∑PCB) and total DDT (∑DDT) in the blubber of male and female bottlenose whales from this study in comparison to concentrations reported for the blubber of other odontocetes from the North Atlantic

Species	Location	*N*	% Lipid	∑PCB[a]	∑DDT[b]	Conc. units	Source
Northern bottlenose whale, *Hyperoodon ampullatus*	The Gully 1996–1997	5m	40.4	7270	14,660	Lipid	This study
		5f	66.3	3790	4140		
	The Gully 2002–2003	6m	50.9	6840	21570	Lipid	This study
		4f	43.2	4060	7600		
	Davis Strait 2003	2m	44.6	3560	3580	Lipid	This study
		1f	38.3	530	1080		
Cuvier's beaked whale, *Ziphius cavirostris*	Bermuda	3m	–	9610	31,190	Wet weight	Knap and Jickells (1983)
		1f	–	9030	12,250		
Blainville's beaked whale, *Mesoplodon densirostris*	UK	1f	83	4780	6150	Wet weight	Law et al. (1997)
	USA	2m	–	14,000–29,000	38,200–65,100	Wet weight	Taruski et al. (1975)
Sperm whale, *Physeter macrocephalus*	Iceland	10m	40.3	10,510	7800	Lipid	Borrell (1993)
	NW Spain	8m	66.4	9930	5100	Lipid	Aguilar (1983)
		6f	68.9	15,550	7730		
Pilot whale, *Globicephala melas*	Cape Cod, USA	21m and f	–	7550	18,340	Lipid	Weisbrod et al. (2000)
Narwhal, *Monodon monoceros*	Canadian arctic	15m	–	5180	5920	Wet weight	Muir et al. (1992)
		6f	–	2700	2540		
Beluga whale, *Delphinapterus leucas*	Greenland	71m	–	1800–10,900	1000–8100	Wet weight	Stern et al. (1994)
		67f	–	800–7200	400–5400		

All totals are shown in ng/g. Either means or ranges are provided. *N* = number of animals, m = male, f = female.
[a] ∑PCB calculated for various numbers of congeners in each study.
[b] ∑DDT calculated from 4,4′- and 2,4′-isomers for this study and Weisbrod et al. (2000); ∑DDT calculated from only 4,4′-isomers for all other studies.

caused by metabolism. It is also unlikely that these whales changed their feeding patterns or geographic range. A study of the diving behaviour of bottlenose whales in The Gully showed that these whales consistently performed deep dives to the sea-floor, presumably to feed on deep-sea squid (Hooker and Baird, 1999; Hooker et al., 2001b). Work on the ranging behavior of individual whales suggests that they are relatively site-faithful (Dalebout et al., 2001; Whitehead et al., 1997; Wimmer and Whitehead, 2004). They do not appear to travel widely and obtain their food from a local source; in fact, some form of territoriality may even be present (Hooker et al., 2002). There have been no observed changes in foraging or ranging behaviour that might drive such difference in contaminant load (personal observations by S.H. and H.W.). The change in contaminant levels over time in these whales is therefore more likely to reflect a temporal change in contaminant levels in the water and/or in prey species. This could be due to alterations in the patterns of oceanic currents, but it cannot be ruled out that the proximity of oil and gas drilling activities may have influenced contaminant patterns. These organochlorine contaminants are not likely to have been released directly from oil rigs or through seismic exploration, but it is possible that oil and gas activities have led to remobilization of persistent contaminants from sediments on the Scotian Shelf.

Changes in CYP1A1 expression in Gully samples did not show a consistent pattern of increase between 1996–1997 and 2002–2003, in that scores of whales sampled in 2002 showed no difference from those sampled in 1996 and 1997. However, samples in 2003 showed significantly higher CYP1A1 expression than observed during other years of sampling in The Gully (Fig. 2). Coincident with this were several spills of kerosene and streamer fluids during seismic survey work conducted during June–August, 2003 (personal communication from Canada Nova Scotia Offshore Petroleum Board). A total of 10 spills of kerosene and streamer fluids were reported, ranging in size from 0.008 to 0.5 cubic metres. Four spills occurred during June, and three in each of July and August. At no time were slicks observed, but fluid was recorded missing when streamers were recovered. Whether such streamer fluids contained compounds which induce CYP1A1 which were not recorded in the blubber contaminants (such as PAHs) is unknown.

4.3. Differences between contaminant levels in The Gully and in the Davis Strait

Blubber contaminant concentrations in northern bottlenose whales from the Davis Strait were significantly lower than concentrations in The Gully whales. This difference could simply reflect differences in the oceanic distribution of contaminants on the Scotian Shelf relative to the Davis Strait in the eastern arctic, consistent with general observations of elevated contaminant exposure at mid-latitudes that is associated with nearby industrialization (Aguilar et al., 2002). However, despite a relatively small sample size, levels of CYP1A1 expression were consistently higher within the three animals

sampled from the Davis Strait population in comparison to those sampled from The Gully population.

Elevated CYP1A1 expression could theoretically reflect mobilization of blubber fats and the coplanar PCBs therein. If this was occurring, it might suggest that northern bottlenose whales in the Davis Strait were undergoing a period of poor feeding. However, we have no supporting evidence for this hypothesis. Alternatively, the higher levels of CYP1A1 exposure in the Davis Strait could be due to exposure to compounds (such as PAHs) which cause CYP1A1 expression, but cannot be detected in tissue. PAHs are rapidly metabolized in marine mammals and are generally present at low to non-detectable concentrations in the tissues of odontocetes (Law and Whinnett, 1992), but studies of beluga whales from the highly contaminated St. Lawrence region have shown evidence of PAH exposure through the presence in tissues of aromatic DNA adducts (Ray et al., 1991). Exposure to PAHs near oil and gas drilling operations is poorly understood and may be a cause for concern. It is possible that there are elevated levels of one or more compounds that interact with the Ah-receptor (e.g., coplanar PCBs, halogenated dioxins and dibenzofurans) that were not quantified in our analysis of blubber contaminants. Elevated levels of persistent contaminants, primarily PCBs have been observed in the arctic, associated with military sites such as the radar station at Saglek Bay, Labrador (approximately 250 km from sampling locations) (Kuzyk et al., 2005). CYP1A activity has been found to be relatively high in the tissues of polar bears in the Canadian Arctic (Muir et al., 1999), and these authors suspected that non-*ortho* and mono-*ortho* PCBs were responsible for this activity. There has been recent industrial activity in Voisey's Bay (35 km southwest of Nain, Labrador), where nickel–copper–cobalt mining began in 2004. However, this is over 200 km from the region where the northern bottlenose whales were sampled and their deep-water habitat, and so, unless there have been major spills, this is unlikely to have caused a large impact on these whales.

5. Conclusions

Blubber contaminants found in biopsies from free-ranging northern bottlenose whales from eastern Canada showed concentrations and patterns consistent with those reported for other North Atlantic cetaceans. These levels were below those suspected of causing health problems in more contaminated odontocetes which is reassuring in the assessment of an ''endangered'' population of whales in a Marine Protected Area. However, blubber contaminant concentrations were higher for whales sampled in The Gully than for those sampled in the Davis Strait. Furthermore, contaminant levels in The Gully population increased dramatically between the 1996–1997 and 2002–2003 time periods for 4,4′-DDE and *trans*-nonachlor, although whether this increase was related to the onset of nearby drilling for gas is unknown.

Northern bottlenose whales do not generally appear to show high expression of CYP1A1, nor they are particularly sensitive to CYP1A1 inducers compared to other odontocetes. However, there was a significant difference in CYP1A1 expression observed in 2003 for The Gully population compared to that observed in 1996, 1997 or 2002. Since this was the final year of sampling, it is not known whether this was a single year event, or whether this may have continued beyond 2003. Our results also suggest that animals in the Davis Strait may be exposed to higher concentrations of CYP1A1 inducing contaminants than Gully animals. Repeated sampling of animals in both The Gully and the Davis Strait would be useful to help resolve these questions.

Differences between CYP1A1 results and blubber contaminants demonstrate the utility of the comparison between these two techniques. Both have highlighted potential concerns for the Gully Marine Protected Area due to development of the oil and gas industry.

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