# USING ANTHROPOGENIC CONTAMINANTS AND STABLE ISOTOPES TO ASSESS THE FEEDING ECOLOGY OF GREENLAND SHARKS

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Abstract. Organochlorine contaminants (OCs) are a large group of ubiquitous pollutants that have potential as tracers of ecological processes. To examine this utility, we measured OCs, stable isotopes of nitrogen ( $\delta^{15}N$ ) and carbon ( $\delta^{15}C$ ), and stomach contents in a large Arctic marine fish, the Greenland shark (Somniosus microcephalus), collected in the Davis Strait region to examine the feeding ecology of this little studied elasmobranch. Stable isotopes and OCs were also measured in the turbot (*Reinhardtius hippoglossoides*) and stable isotopes in the ringed seal (Phoca hispida) and harp seal (Pagophilus groen*landicus*) to put the shark results in context. Values of  $\delta^{15}N$  suggest that the Greenland shark feeds at a similar trophic level as the turbot and ringed seal (about the fourth trophic level) and at a higher trophic level than harp seals, despite the presence of many turbot and a single ringed seal in the stomach contents of 14 sharks. Values of  $\delta^{13}$ C indicate that source of carbon in turbot and Greenland shark is of a more pelagic origin than in ringed and harp seals. High concentrations of biomagnifying OCs in the sharks compared with the turbot (concentration  $10-100 \times \text{lower}$ ) and ringed seals ( $3-10 \times \text{lower}$ ) suggest that the sharks feed at a higher trophic level than implied by stable isotopes. High urea levels found in the tissues of sharks may influence  $\delta^{15}$ N values, resulting in an underestimate of shark trophic position, and requires additional study. The presence of a ringed seal in the stomach of one shark, relatively high levels of a contaminant metabolite (oxychlordane; slowly formed in fish) in some sharks, and high OC levels suggest that seals may be a common food item of some Greenland sharks. This study shows the utility of using OCs in ecological study and suggests caution when interpreting stable-isotope data as a single indicator of trophic position.

Key words: arctic; elasmobranchs; feeding ecology; Greenland shark; marine mammals; organochlorine contaminants; pollutants; seals; stable isotopes; trophic position.

## INTRODUCTION

The diet and trophic position are important components in understanding the ecology of a species and for the management of important commercial animals. Although trophic relationships in terrestrial systems can be simple, these relationships in aquatic food webs, in particular marine food webs, can be complicated and difficult to assess. This can be particularly true for rare or remote (e.g., deep water or polar) species, which can be difficult to collect.

Stomach contents have long been used to assess diet and assign trophic level in aquatic food webs (see Cortes 1997). However, unless a very comprehensive sampling regime is used, stomach contents can be misleading and provide only a small snapshot of a species diet. Different rates of digestion of prey species, seasonal changes, and uncommon prey items may all confound conclusions based on stomach contents, particularly at low sample sizes. Stable isotopes of nitrogen and carbon methods reduce many of the uncertainties of stomach content analysis (Hansson et al. 1997, Vander Zanden et al. 1997). The ratio of the heavier to lighter stable isotopes of nitrogen (15N:14N), expressed relative to a standard as  $\delta^{15}N$ , generally increases with trophic position in aquatic food webs, providing a continuous variable with which to assess trophic level (Vander Zanden et al. 1997). Stable-carbon isotope ratios (13C:12C) show less enrichment with trophic transfer but can be useful in evaluating sources of primary production in marine systems as well as general patterns of inshore/benthic vs. offshore/pelagic feeding preferences (France 1995, Lawson and Hobson 2000). Stable isotopes provide a number of advantages in assessing trophic position and carbon source because they are easily determined and incorporate diet over a longer period than stomach contents. However, there are limitations and potential problems when using stable isotopes. Due to the limited number of stable-isotope variables measured (generally isotopes of N and C; although H, S, and O can increase this number), it is often difficult or not possible to make conclusions on

Manuscript received 6 November 2001; accepted 5 December 2001; final version received 3 January 2002.

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August 2002

type and rate of consumption of prey items, particularly when a large number of prey items with similar stableisotope values are present. There are only a limited number of studies examining the turnover of stable isotopes in different species (Gannes et al. 1997) and often a single rate is used across mainly types of organisms, from invertebrates to mammals (Hobson et al. 1995, 2001), potentially leading to inaccurate conclusions on trophic position.

Organochlorine contaminants (OCs) are a large class of ubiquitous anthropogenic chemicals (e.g., PCBs and DDTs) that have been used as pesticides and industrial products since the end of World War II. Due to their high persistence and toxicity, they have been the focus of much research and therefore much is understood about their fate and dynamics in the environment (Fisher 1995), including the Arctic (Arctic Monitoring and Assessment Programme [AMAP] 1998). The historical use and environmental behavior of OCs is quite varied and often their resultant concentrations and relative proportions in biota can provide insights into the biology of species. Although infrequent, there are examples of OCs being used to trace ecological processes. For example, Hebert and Haffner (1991) showed that the concentration of OCs in four species of forage fish was related to habitat partitioning, with greater concentrations of OCs in benthic fish, and Muir et al. (1995) showed that high concentrations of PCBs in some populations of walrus (Odobenus rosmarus) were likely related to predation on seals.

Assessing diet and trophic position in rare or difficult-to-collect species is a problem. This is obvious in Arctic and deepwater fish species where the logistics of field collections are difficult and costly and the commercial fisheries are limited and not easily utilized. This is exemplified by the Greenland shark, Somniosus microcephalus, a large fish, up to 7.3 m (Compagno 1984) and 775 kg (Coad 1995), which inhabits deep and/or Arctic waters (see Plate 1). Very little is known about Greenland shark biology, yet limited evidence indicates that these animals may be long lived (>100 yr; see Hansen 1963), potentially numerous (Coad 1995), and may play an important, but unstudied, role in Arctic marine food webs. For example, there is evidence that Greenland sharks feed on marine mammals, in particular Arctic seals, including ringed seals (Phoca hispida; Ridoux et al. 1998; M. Treble, personal communication), harp seals (Phoca groenlandica), and bearded seals (Erignathus barbatus; M. Treble, personal communication).

To assess the feeding ecology of Greenland sharks, and the use of OCs as tracers of feeding ecology, OCs, stable isotopes of nitrogen and carbon, and stomach contents were determined for Greenland sharks collected in the Canadian Arctic. Stable isotopes and OCs were also determined in turbot (*Reinhardtius hippoglossoides*, also known as Greenland halibut), a prey item of *S. microcephalus*, and stable isotopes in ringed



PLATE 1. Male Greenland shark (total length 269 cm) captured 5 June 1996 in Victor Bay, Northwest Territories, Canada. Photograph by George W. Benz.

seal (*Phoca hispida*) and harp seal (*Pagophilus groenlandicus*) to provide additional information for comparison with Greenland sharks.

# METHODS

### Sample collection and stomach content analysis

Fifteen Greenland sharks (five females and 10 males) and four turbot (two females and two males) were collected in April 1999 from Cumberland Sound (65°58' N, 66°41' W) using longline fishing techniques, with Arctic charr (Salvelinus alpinus) as bait, through the sea ice in water  $\sim$ 554 m deep. Sharks and turbot were removed from the water and fork lengths (cm) and sex were measured. OCs are very hydrophobic and accumulate in fatty tissue and therefore livers were chosen for analysis because of their high lipid content in sharks. Muscle tissue was chosen for the turbot because the muscle makes up the largest percentage of tissue and would reflect the exposure to OCs by the shark upon consuming a turbot. Liver samples ( $\sim$ 50 g) were taken from the left lobe 30 cm from the anterior end in the shark, and muscle samples were taken from a point middle line and to the left of the vertebrae in the turbot, placed in a Whirl-Pak bag (Nasco, Ft. Atkinson,

TABLE 1. Length,  $\delta^{15}N$ , and  $\delta^{13}C$  values (mean  $\pm 1$  SE) in Greenland shark liver and turbot muscle collected in 1999 from Cumberland Sound and in Greenland sharks collected in Davis Strait in 1997.

Sample	n	Length (cm)	$\delta^{15}N$	$\delta^{13}N$
Cumberland Sound Turbot Greenland shark	4 15	$60.8 \pm 6.02$ 283.6 ± 5.7		$-20.5 \pm 0.4$ -19.9 $\pm 0.1$
Davis Strait Greenland shark	2	135–135	12.2-15.1	-19.9520.62

Wisconsin, USA) and were kept frozen until analyzed. Stomachs were opened and the contents were emptied onto the packed snow above the sea ice. The types of organisms found were noted and organisms larger than brittle stars (i.e.,  $>\sim5$  g) were quantified. In addition, two sharks were collected in boat-hauled trawls in the Davis Strait to the east of Cumberland Sound (exact coordinates unknown) during September 1997. These sharks were frozen whole and measurements and tissue samples were collected back in the laboratory, but no sex data were recorded. Samples of harp and ringed seal muscle collected from Cumberland Sound in 1999 were provided from a separate study.

## Stable-isotope analysis

Liver (shark) or muscle (turbot) samples were thawed, subsampled (0.5-1.0 g), and freeze-dried for  $\sim$ 24 h. Lipids were removed for consistency with past studies on stable isotopes in Arctic marine food webs (Hobson et al. 1995, 2001). To remove lipids, the samples were homogenized in toluene using a Tekmar TR-10 ultraturrax (Tekmar Dohrmann, Mason, Ohio, USA) and centrifuged using a Sorvall angle centrifuge (model NSE; Kendro, Asheville, North Carolina, USA) at 3000 RPM for 3 min. Toluene is a very nonpolar solvent and has been used previously to remove lipids from biotic tissue (Fisk et al. 1996). The supernatant (toluene and lipid) was decanted and the samples left to dry overnight in a fume hood. Stable carbon and nitrogen isotope ratios were determined by combustion on a CE Elemental analyzer (CE Instruments, Milan, Italy). Carbon and nitrogen were separated by gas chromatography by continuous flow on a Finnigan MAT Delta<sup>plus</sup> IRMS (isotope ratio mass spectrometer; Thermo Finnigan, Breman, Germany). The gases from the samples were compared in the mass spectrometer to air (for  $N_2$ ) and VPDB (PeeDee Belemnite, for  $CO_2$ ). Carbon standards used to normalize the data included NBS No. 21 spectroscopic graphite (National Biological Survey, Washington, D.C., USA), USGS-24 graphite isotopic reference material (U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, Maryland, USA), and IAEA-CH-6 sucrose No. 173 (International Atomic Energy Agency, Vienna, Austria). Nitrogen standards used included IAEA-N-1 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> No. 246, IAEA-N-2 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> No. 245, and USGS-25  $(NH_4)_2SO_4$  No. 12. The precisions for carbon and nitrogen analyses were  $\pm 0.30\%$  and  $\pm 0.20\%$ , respectively.

### Organochlorine analysis

Samples were prepared using a method similar to that described in Norstrom et al. (1988). Briefly, accurately weighed samples of turbot muscle ( $\sim 10$  g, skin on) or shark liver ( $\sim 5$  g) were mixed with activated Na<sub>2</sub>SO<sub>4</sub> until a free-flowing mixture was obtained. After addition of an internal recovery standard consisting of six <sup>13</sup>C<sub>12</sub>-labeled PCB congeners, the mixtures were wet packed with dichloromethane/hexane (1:1 by volume) into a glass column (2 cm inner diameter). Lipid and lipid-soluble contaminants were extracted with 250 mL of dichloromethane/hexane (1:1) eluant. After extraction, the lipid content of the samples was determined gravimetrically. Lipids were then removed by gel permeation chromatography (GPC). The lipid-free GPC fractions were reduced to  $\sim 1$  mL and applied to a Florisil column (8 g, 1.2% deactivated; Floridin, Berkeley Springs, West Virginia, USA). OCs were eluted using 110 mL of dichloromethane/hexane (1:1). The Florisil column eluates were reduced to 500 µL after addition of 1 mL of isooctane to the eluates. Prior to analysis, <sup>13</sup>C<sub>12</sub>-CB138 was added to all samples and standards as a volume corrector.

OCs were analyzed using a Hewlett Packard 5987B mass spectrometer (Hewlett Packard, Wilmington, Delaware, USA) coupled via a 5988 GC/MS direct interface to a 5890 Series II GC (Hewlett Packard, Wilmington, Delaware, USA). The GC was equipped with a 30 m  $\times$  0.25 mm inner diameter DB5-MS column (J&W Scientific, Folsom, California, USA) with a film thickness of 0.25 µm. Volumes of 2.0 µL were injected in the splitless mode using a Hewlett Packard 7673 autoinjector (Hewlett Packard, Wilmington, Delaware, USA). All compounds were analyzed in the electron impact ionization single-ion-monitoring mode. PCBs were quantitated using an external standard containing equal amounts of Aroclors 1242, 1254, and 1260 (Accustandard, Brockville, Ontario, Canada). Other organochlorine compounds were quantitated in a similar fashion using external standards. The level of detection varied with the compound and the amount of tissue extracted, but was  $\sim 0.1$  pg/g. Compounds that were not detected in a sample were assigned a value of 0. The percentage of recovery of <sup>13</sup>C-PCB internal standards was 75  $\pm$  1.4%. Concentrations were corrected for recovery.

### Statistical analysis

Values of  $\delta^{15}$ N and  $\delta^{13}$ C in the Greenland sharks, turbot, harp seals, and ringed seals were normally distributed and therefore no transformation was preformed on this data. The sex of the two Davis Strait Greenland sharks was not recorded, and therefore the influence of sex and fork length on stable-isotope values of Greenland shark was first assessed in the Cumberland Sound samples only using the general linear model

$$\delta X = \mu$$
 + fork length + sex  
+  $\mu Y$  + (fork length × sex) +  $\varepsilon$ 

where  $\delta X$  is  $\delta^{15}$ N or  $\delta^{13}$ C and  $\delta Y$  is the opposite stableisotope parameter,  $\mu$  is a constant, and  $\varepsilon$  is an error term. The significance of each variable was then assessed with Type-III sum-of-squares test. All GLM analyses were preformed using SAS (SAS Institute 2000). Sex was not found to significantly influence stable-isotope values (P > 0.5), and therefore the data from the two smaller sharks from Davis Strait were included for an assessment of the influence of fork length of stableisotope values using the model

$$\delta X = \mu + \text{fork length} + \delta Y + \varepsilon$$

where  $\delta X$  is  $\delta^{15}$ N or  $\delta^{13}$ C and  $\delta Y$  is the opposite stableisotope parameter,  $\mu$  is a constant, and  $\varepsilon$  is an error term. The significance of each variable was then assessed with Type-III sum-of-squares test.

Differences in stable-isotope values between Greenland sharks (n = 15), turbot (muscle, n = 4), harp seals (muscle, n = 6), and ringed seals (muscle, n = 6) collected in Cumberland Sound in 1998 were assessed with ANOVA and an a posteriori Tukey's test using SYSTAT (SPSS 1999).

Due to the limited number of shark samples, statistical analysis of OCs were limited to  $\Sigma PCB$  (the sum of PCBs),  $\Sigma$ DDT (the sum of DDT), and oxychlordane.  $\Sigma$ PCB was chosen because it was the most abundant OC,  $\Sigma$ DDT and oxychlordane were chosen because they were used in additional statistical analyses. All OCs concentrations were lipid normalized (concentration/lipid percentage) prior to statistical analysis due to the variability of lipid content between sharks. OC concentrations were found to be normally distributed and no transformations were performed. As with stable isotopes, sex was not found to influence OC concentrations (P > 0.05) in the Cumberland Sound sharks using a GLM. The influence of fork length,  $\delta^{13}$ C, and  $\delta^{15}$ N on OC concentrations in all Greenland shark (n = 17) samples were assessed using the general linear model

$$OC = \mu + \text{ fork length} + \delta^{13}C + \delta^{15}N + \varepsilon$$

where OC is either  $\Sigma$ PCB,  $\Sigma$ DDT, or oxychlordane,  $\mu$  is a constant, and  $\varepsilon$  is an error term. The significance

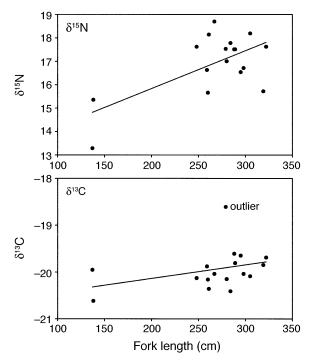


FIG. 1. Relationship between fork length (cm) and  $\delta^{15}$ N (top graph;  $\delta^{15}$ N = 12.66 + 0.016(fork length),  $r^2 = 0.42$ , P = 0.005) and  $\delta^{13}$ C (bottom graph; outlier removed,  $r^2 = 0.23$ , P = 0.06) in Greenland sharks. Note that the two smallest sharks were collected in Davis Strait, and all others were collected in Cumberland Sound.

of each variable was then assessed with Type-III sumof-squares test.

Relationships between the ratio of oxychlordane to  $\Sigma$ chlordane (the sum of all chlordanes) and concentrations of OCs were assessed using linear regression using SYSTAT (SPSS 1999). Ratios of concentrations of OCs on a lipid weight basis between Cumberland Sound Greenland shark (CSGS) and turbot were calculated for comparison to biomagnification factors (BMFs) found in other marine food webs, based on the assumption that turbot are representative of the sharks diet. BMFs were calculated with lipid corrected concentrations using the equation (Fisk et al. 2001*a*) BMF = (shark OC concentration)/(turbot OC concentration). Concentrations are measured as ng OC/g tissue and are lipid normalized.

# RESULTS

### Stable isotopes and stomach contents

Stable-isotope values for the Greenland shark and turbot are summarized in Table 1. Sex and fork length were not significant variables influencing  $\delta^{15}$ N or  $\delta^{13}$ C (GLM, df = 5, 9, P > 0.05) in the Cumberland Sound Greenland shark. The inclusion of the two smaller Greenland sharks from Davis Strait resulted in fork length being a significant variable influencing  $\delta^{15}$ N (GLM, df = 2, 14, P< 0.04) but not  $\delta^{13}$ C (GLM, df = 2, 14, P > 0.05; Fig. 1). The influence of sex could not be assessed with the

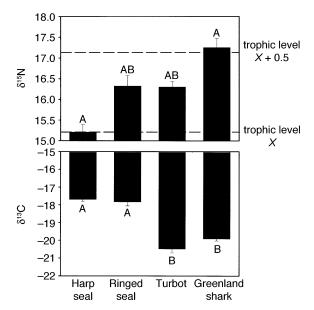


FIG. 2. Values of  $\delta^{15}$ N (top graph; mean +1 sE) and  $\delta^{13}$ C (bottom graph; mean -1 sE) in ringed and harp seals (muscle, n = 6 for both species), turbot (n = 4, muscle), and Greenland shark (liver, n = 15) collected in Cumberland Sound in 1999. Different letters indicate that means are significantly different at P < 0.05. Dashed lines represent the trophic level and the trophic level +0.5, assuming that one trophic level is 3.8‰  $\delta^{15}$ N (Hobson et al. 1995).

larger data set because the sexes of the Davis Strait sharks were not known. No relationship was found between  $\delta^{15}N$  and  $\delta^{13}C$  in the Greenland sharks (GLM, df = 2, 14, P > 0.05).

The values of  $\delta^{15}$ N in Cumberland Sound Greenland sharks were found to be significantly greater than harp seals (ANOVA, df = 3, 27, *P* > 0.05) but not turbot or ringed seals (ANOVA, df = 3, 27, *P* < 0.05; Fig. 2).  $\delta^{15}$ N values did not vary significantly between turbot, ringed seals, or harp seals (ANOVA, df = 3, 27, *P* > 0.05; Fig. 2).  $\delta^{13}$ C values did not vary between turbot and Greenland shark (ANOVA, df = 3, 27, *P* > 0.05) or between harp seals and ringed seals (ANOVA, df = 3, 27, *P* > 0.05), but the fish were significant depleted in <sup>13</sup>C as compared with the mammals (Fig. 2).

Stomach contents of the Cumberland Sound Greenland sharks were predominantly benthic organisms (Table 2). The designation of benthic or pelagic were based on the habitat of the organism. Stomach contents of the Davis Strait sharks were not determined. Stomachs of the turbot were generally empty but there were remnants of large zooplankton (mysids or shrimp) in one turbot and fish in another.

# OC concentrations

Concentrations of OCs in the Greenland shark and turbot along with other upper trophic level organisms from other Arctic studies are summarized in Table 3 and Fig. 3. Sex was not found to be a significant variable describing the OC concentrations in the Cumberland Sound sharks (GLM, df = 5, 9, P > 0.05). Fork length,  $\delta^{15}$ N, or  $\delta^{13}$ C were not found to significantly influence OC concentrations in all Greenland sharks (GLM, df = 5, 11, P > 0.05). OC concentrations were greater in the larger sharks of Cumberland Sound compared with the Davis Strait sharks, however, due to the limited number of Davis Strait sharks (n = 2), no statistical comparison was performed.

# DISCUSSION

This study provides strong data that the Greenland shark is an upper trophic level organism, consistent with past suggestions in literature (Coad 1995). However, the methods used, stable isotopes, concentrations of OCs, and stomach contents, were not in complete agreement.

 TABLE 2.
 Stomach contents of Greenland sharks from Cumberland Sound.

Stomach content	Benthic/ pelagic	Occurrence (%) (N = 14)	
Invertebrates <sup>†</sup>			
Squid beaks	pelagic	7	
Jellyfish	pelagic	7	
Sunstar	benthic	7	
Brittle star	benthic	7	
Amphipods	benthic	14	
Marine snails	benthic	21	
Fish§			
Arctic eelpout	benthic	7	
Arctic charr	pelagic	7 (+7)	
Grenadier	benthic	7	
Skate jaws	benthic	7	
Greenland shark flesh		7	
Turbot	benthic	71	
Unknown fish meat		29	
Mammals			
Ringed seal	pelagic	7	
Unknown mammal		7	
meat (not seal)¶			
Total invertebrate			36
Total fish			100
Total mammal			14
Total benthic‡			100
Total pelagic‡			23

*Notes:* N = 14 (the stomach of one shark had been eaten by another). Note that many of the turbot in the shark's stomachs were most likely eaten off the longlines (hooks were found in 3 of 14 stomachs). The designation of benthic or pelagic was based on the habitat of the organism.

† Invertebrates were not identified to species.

 $\ddagger N = 12$  for total benthic and pelagic values (two sharks only had unidentified fish remains that could not be counted as either benthic or pelagic).

§ Fish were not identified to species with the exception of Arctic charr (*Salvelinus alpinus*).

 $\|$  Charr bait from the longlines was found in the stomach of one shark.

¶ The unknown mammal meat was identified as nonseal by Inuit fisherman and hunter David Evic (Pangnirtung, Nunavit, Canada). This tissue was likely scavenged off the bottom due to the presence of the scavenging amphipod *Anonyx nugax* throughout the tissue.

TABLE 3. Organochlorine contaminant concentrations (ng/g, lipid basis) in selected Arctic biota of Cumberland Sound and northern Baffin Bay.

Species, year	ΣClBz†	ΣHCH‡	ΣCHLOR§	$\Sigma DDT \parallel$	ΣPCB¶	Reference
Cumberland Sound						
Turbot, 1999	$55 \pm 4.4$	$81 \pm 5.5$	$162 \pm 18$	$78 \pm 7.4$	$58 \pm 12$	this work
Greenland shark, 1999	$52 \pm 46$	$53 \pm 6.2$	$1815 \pm 273$	7159 ± 1271	$3442 \pm 650$	this work
Ringed seal, 1993	38	220	493	738	704	Muir (1994)
Beluga, 1993–1994	720	479	3815	6065	6176	Muir et al. (1999)
Polar bear, 1993-1994	576	454	2200	7186	8816	Fisk et al. (2001b)
Northern Baffin Bay Glaucous Gull, 1998	NA	NA	2315	213	8037	Norstrom et al. 1998

*Notes:* Turbot (n = 4) and Greenland shark (n = 15) values are mean  $\pm 1$  sE.  $\pm \Sigma$ ClBz (chlorobenzene) = sum of 1,2,4,5-tetraClBz, 1,2,3,4-tetraClBz, pentaClBz and hexaClBz.

 $\ddagger \Sigma$ HCH (hexachlorocyclohexane) = sum of  $\alpha$ -HCH,  $\beta$ -HCH and  $\gamma$ -HCH.

 $\Sigma$ CHLOR (chlordanes) = sum of heptachlor, heptachlor epoxide, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*nonachlor, and oxychlordane.

 $\| \Sigma DDT = \text{sum of } p, p' \text{-}DDD, p, p' \text{-}DDE \text{ and } p, p' \text{-}DDT.$ 

¶ ΣPCB = sum of congeners 1, 3, 4/10, 7, 6, 8/5, 19, 18, 17, 24/27, 16/32, 26, 25, 31, 28, 33, 22, 45, 46, 52, 49, 47, 48, 44, 42, 41/71, 64, 40, 74, 70/76, 95/66, 56/60, 91, 84/89, 101, 99, 83, 97, 87, 85, 136, 110, 82, 151, 144/135, 149, 118, 134, 114, 131, 146, 153, 132, 105, 141, 130/176, 179, 137, 138, 158, 178/129, 175, 187, 183, 128, 185, 194, 196/203, 189, 208, 195, 207, 194, 205, 206, and 209.

Values of  $\delta^{15}N$  suggested a trophic level that was the same as turbot and ringed seals but both of these organisms were found in the stomachs of the Greenland shark. In addition, concentrations of biomagnifying OCs were much higher in the Greenland sharks than the turbot or ringed seals, indicating a much higher trophic position. This study highlights the potential problems and limitations of using  $\delta^{15}N$  as the only surrogate for trophic position.

Values of  $\delta^{15}N$  in the Greenland shark were not statistically different than those observed in the ringed seals and turbot, suggesting a similar trophic level. The trophic level of ringed seals has been shown to be slightly

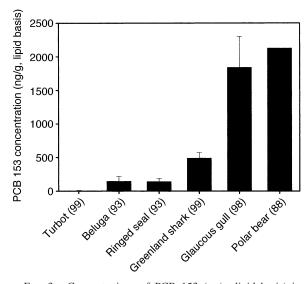


FIG. 3. Concentrations of PCB 153 (ng/g, lipid basis) in turbot, beluga, ringed seal, Greenland shark, Glaucous Gull (collected in northern Baffin Bay), and polar bear from Cumberland Sound (see Table 3 for references). Year of collection is provided in parentheses (99 = 1999, etc). Values are mean +1 sE.

higher than fourth based on  $\delta^{15}N$  values determined for an Arctic marine food web collected to the north of the present study site (Hobson et al. 2001).  $\delta^{15}$ N is positively correlated with trophic level with an approximate change of 3.8‰, often termed an enrichment factor, per trophic level (Michener and Schell 1994, Hobson et al. 1995). The  $\delta^{15}N$  values in the turbot are similar to values in ringed and harp seals and were not unexpected as these fish feed predominantly on other fish and large zooplankton (e.g., mysids and shrimp), similar to ringed seals (Holst 2000), and even on their own species (Bowering 1993).

The similar  $\delta^{15}$ N values between the shark, turbot, and ringed seals do not agree with the stomach contents of the Greenland sharks found in this study and past studies. Turbot were the most common prey item found in the stomachs of the larger sharks (Table 2) although this may have been an artifact of the longline sampling methods, which provide a large number of hooked turbot as easy prey. Hooks were found in the stomachs of some sharks, suggesting that this was indeed the case, although it should be noted that turbot have been identified as a prey item of Greenland shark (Coad 1995, Ridoux et al. 1998). Although only one of 14 shark stomachs examined contained seal tissue. This finding is significant, particularly in light of previous observations of seals in the stomachs of Greenland sharks (Ridoux et al. 1998; M. Treble, personal communication). The Greenland sharks collected for this study were captured on the bottom at a depth of  $\sim$ 554 m, well below the maximal depth for ringed seals pups (Lydersen and Hammill 1993), and longline captured sharks may have been targeting turbot instead of seals at this time. The collection time of this study was near the end of the ringed seal pupping season (McLaren 1958) and possible seal predation/consumption by sharks may increase with

TABLE 4. Biomagnification factors (BMFs) for organochlorine contaminants (OC) in Cumberland Sound Greenland sharks using turbot as a prey item.

Contaminant	BMFs
ΣClBz ΣHCH	6.3 0.7
oxychlordane	47
ΣCHLOR	11
<i>p,p-</i> DDE <i>p,p-</i> DDT	62 183
ΣDDT	92
PCB 153 PCB 138/163 PCB 101/90 PCB 118 PCB 99	64 144 30 40 43
ΣΡCB	60

*Notes:* All concentrations were lipid corrected. The five major OC groups, individual OCs used in the discussion, and the five PCB congeners with the high concentrations in the sharks are presented.

time as mother seals spend more time at their lairs and young seals spend more time in the water.

Concentrations of OCs in the Greenland shark were in the range of other top Arctic marine predators, and well above those found in ringed seals (Table 3, Fig. 3), and provide evidence that these sharks feed at a trophic level higher than suggested by  $\delta^{15}N$ . Concentrations of recalcitrant OCs increase with each step in the food web resulting in the highest concentrations at the top of the food web (Muir et al. 1988, Fisk et al. 2001a). OCs that can be metabolized do not biomagnify. For example, polar bears (Ursus maritimus) and Glaucous Gulls (Larus hyperboreus) represent the top of the Arctic marine food web (Hobson et al. 1995). This is reflected in their high  $\Sigma$ PCB concentrations created by food web biomagnification and the limited biotransformation of these compounds (Muir et al. 1988, Fisk et al. 2001a).  $\Sigma$ PCB,  $\Sigma$ chlordanes, and  $\Sigma$ DDT concentrations, all of which biomagnify in Arctic marine food webs (Fisk et al. 2001a), were higher in Greenland sharks than in ringed seals but lower than in polar bears and Glaucous Gulls (Table 3). There is also strong evidence that homeotherms accumulate much higher OC concentrations than poikilotherms because of much higher feeding rates in homeotherms (Fisk et al. 2001a). Therefore, the high OC concentrations in the Greenland sharks suggest a trophic position well above ringed seals, which is consistent with the concentration of PCB 153 in Arctic biota (Fig. 3), a very recalcitrant compound in all species, often used as a marker for biomagnification (Boon et al. 1989).

Biomagnification factors (BMFs) calculated for the Greenland shark, using turbot as the prey item, were very high for a fish species. These BMFs in the sharks ranged from  $\sim 1$  for the lesser chlorinated compounds to >100 for some PCB congeners and p,p'-DDT (Table

4). BMFs for recalcitrant OCs, such as PCBs, are generally much lower in fish (PCB BMFs <10) than in mammals and birds (PCB BMFs >20; Muir et al. 1999, Fisk et al. 2001a). These results suggest that turbot do not represent the major source of OCs to sharks and that the sharks are consuming a more contaminated prey item. There are a number of potential explanations for these high BMFs, none of which are conclusive or exclusive. First, the sharks may be at a higher trophic level than suggested by  $\delta^{15}N$  values, potentially more than one trophic level higher than turbot, and therefore the comparison of sharks to turbot to determine a BMF may not be appropriate. A more detailed discussion of this follows. Second, the consumption of higher trophic level organisms, such as ringed seals, would result in a pulse of higher OCs and  $\delta^{15}N$  values compared with a diet of fish and invertebrates. Although the half-lives of OCs and  $\delta^{15}$ N are unknown in large fish, they are likely >1 yr for OCs (Fisk et al. 1998) but <1 yr in liver for  $\delta^{15}N$ (Hesslein et al. 1993) based on laboratory studies in fish. Therefore, the "memory" of a feeding event on a seal after 6 mo would be observed in high OCs but not likely in high liver  $\delta^{15}N$  values. Such discontinuity between  $\delta^{15}$ N and OC concentrations has also been observed in carrion-feeding seabirds (Fisk et al. 2001a). A third explanation has to due with the potentially long life span of Greenland sharks. Growth rates of Greenland sharks in Arctic waters have been estimated to be <1 cm/yr (Hansen 1963), possibly making a 300 cm shark over 100 yr old. Kidd et al. (1998) found that OC concentrations were greatest in the oldest fish from a population of Arctic charr (Salvelinus namaycush). Unfortunately, there is no method for aging Greenland shark. However, since size and OC concentrations were not correlated in the shark, age may be less of a significant variable in sharks as compared with other organisms.

The enrichment factor used to calculate trophic level from  $\delta^{15}N$  for Greenland sharks merits further consideration in light of the discrepancy of trophic level based on  $\delta^{15}$ N with OC concentrations and stomach contents, and also the physiology of elasmobranchs. The basis for the trophic level– $\delta^{15}N$  relationship is the selective excretion of <sup>14</sup>N. Sharks and other elasmobranchs retain high urea and trimethylamine oxide (TMAO) levels in their tissues for osmoregulatory purpose (Olson 1999), and nitrogen contained in both urea and TMAO could influence  $\delta^{15}N$  estimates. Since it is a metabolically formed waste product, it would seem likely that the nitrogen in urea would be isotopically light compared with other shark tissue components. Thus, high levels of retained urea could result in an artificially low  $\delta^{15}N$ value and an underestimation of the trophic level of Greenland sharks when compared to other organisms that do not retain urea. For example, because we do not have an enrichment factor for urea-retaining organisms a single enrichment factor has to be used for sharks, turbot, and seals. The only study containing stable-isotope data for sharks provides some support for the in-

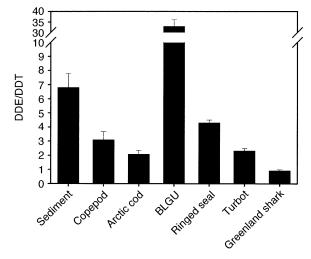


FIG. 4. Ratio of DDE to DDT in turbot and Greenland sharks of Cumberland Sound (this study) and in sediment, copepods, Arctic cod, black gullimot (BLGU), and ringed seals of northern Baffin Bay (A. T. Fisk, *unpublished data*).

fluence of urea on measured tissue values of  $\delta^{15}N$  (Ostrom et al. 1993). This study found that the value of  $\delta^{15}N$  in a single basking shark (*Cetorhinus maximus*) sample was lower than a sample from blue whale (*Balaenoptera musculus*) although both species are planktivores and had similar  $\delta^{13}C$  values. If nitrogen stable isotopes are to be used reliably for trophic studies of elasmobranchs, there is a need for additional research on the influence of urea on measured  $\delta^{15}N$  values as well as laboratory-based determinations of the turnover rates of  $\delta^{15}N$ .

The trophic position of the Greenland shark appears to increase with size based on a positive relationship between  $\delta^{15}N$  and fork length. This observed increase in trophic level with length likely reflects the availability of larger fish as prey, which has been observed in other shark species (Stillwell and Kohler 1982, Lowe et al. 1996), and potentially an increase in predation on marine mammals. It should be noted that the smaller sharks were collected at a different location, Davis Strait, and prev availability may have varied from Cumberland Sound. However, stable-isotope signatures have been shown to be consistent at lower trophic levels within similar regions of the Arctic marine environment (Schell et al. 1998). Since the Davis Strait and Cumberland Sound are geographically close, a comparison of  $\delta^{15}$ N in sharks from these two locations is likely not confounded by differences in  $\delta^{15}N$  at the base of the food webs.

The source of carbon in both the Greenland sharks and turbot appears to be more pelagic than suggested by literature. Both species are considered to be benthic (Coad 1995, Bowering 1993), but  $\delta^{13}$ C values in the shark and turbot were depleted in <sup>13</sup>C when compared to pelagic organisms, such as the ringed and harp seal.  $\delta^{13}$ C is often a good indicator of the source of primary productivity, such as pelagic vs. benthic sources (France

1995). Carbon derived from pelagic sources in the marine environment is generally depleted in <sup>13</sup>C. Ringed seals feed predominantly on Arctic (Boreogadus saida) and polar cod (Arctogadus glacialis) and on pelagic and ice-associated zooplankton (Weslawski et al. 1994, Holst 2000). The relative pelagic  $\delta^{13}$ C signal in the turbot was unexpected based on their benthic nature and diet (Bowering 1993). The  $\delta^{13}$ C signal in the sharks likely reflects a more varied diet; many pelagic components were observed in the stomachs of the sharks (Table 2). Since higher trophic level organisms in aquatic systems have been shown to have intermediate  $\delta^{13}C$  values due to integration of different carbon sources (Hecky and Hesslein 1995), the intermediate  $\delta^{13}$ C signal seen in the sharks and turbot may partially be due to their high trophic position, consistent with their  $\delta^{15}N$ . However, ringed seals had similar δ15N values to the shark and turbot but feed almost exclusively on pelagic prey. The dynamics of nitrogen and carbon stable isotopes in the food web of Cumberland Sound need to be further investigated so that the trophic positions of the Greenland shark and turbot may be properly determined.

OC concentrations (lipid basis) in the Greenland sharks in this study were not related to fork length or  $\delta^{15}$ N. Although length and age have been shown to be positively related to OC concentration in a number of fish species (Miller 1994, Huestis et al. 1996), it was not observed in the shark C. coelolepis, the only other study that has examined OC concentrations and length in sharks (Serrano et al. 2000). Strong relationships between  $\delta^{15}N$  and concentrations of recalcitrant OCs have been reported in Arctic marine food webs (Fisk et al. 2001a), although evidence for this relationship in an individual population or species is limited. The lack of relationships for the Greenland sharks reported here may be due to the limited range in lengths and  $\delta^{15}N$  and the smaller number of samples or possibly also due to variable feeding strategies within shark populations (e.g., some sharks may target ringed seals, of which the effects on OC and  $\delta^{15}$ N are discussed later in this section).

The relative proportions of DDT metabolites in the studied Greenland sharks suggest that this species has a low metabolic capacity and a long life span. DDT is an insecticide that had been widely used but has now been banned in most parts of the world for close to three decades. This has resulted in declining levels of DDT and its metabolites in Arctic biota since the 1970s (Addison and Smith 1998). The relative proportion of DDE in  $\Sigma$ DDT has been increasing with time in Arctic biota from the 1970s until present (Addison and Smith 1998), mainly because DDT is abiotically and biotically degraded to DDE in the environment. DDE is usually the dominant DDT compound measured in Arctic biota (Fig. 4), particularly in birds and mammals that efficiently degrade DDT to DDE. The ratio of DDE to DDT in the Greenland sharks in this study was less than one (Fig. 4). Such a low ratio is usually associated with exposure to a recent use of DDT, such as in Mexico or Africa

(Kidd et al. 2001). This would seem unlikely that an Arctic fish species, which is not believed to migrate great distances (Hansen 1963), could be exposed to recent uses of DDT. Clearly the high proportion of DDT suggests limited biotransformation in the Greenland shark. However, other Arctic organisms that have very limited capability to metabolize DDT, such as zooplankton (Darrow and Harding 1975), that have been collected in the past decade generally have a greater amount of DDE than DDT (Fig. 4). The high DDT in the studied sharks may therefore reflect accumulation of large amounts of DDT in the 1950s through the 1970s when DDT was still used throughout the world and the relative amount of DDT compared to DDE entering the Arctic was higher. It follows that the Greenland sharks sampled in this study were alive during the 1950s through the 1970s, which is altogether possible if the growth rate for Greenland sharks mentioned above is accurate. The lack of a relationship between size, a measure of age, and DDT concentrations suggest that all the sharks examined are of an age that covers this time period.

Concentrations of oxychlordane in the sharks may be an indicator of seal consumption. Oxychlordane is a member of the chlordane class of chemicals. It is not part of the technical mixture used as a pesticide but rather it is a metabolite formed from cis- and transchlordane in seabirds and mammals (Nomeir and Haijar 1987). Although oxychlordane can make up a large percentage of the total chlordane load in seabirds (Fisk et al. 2001b) and mammals (Norstrom and Muir 1994) it is generally nondetectable or found at very low concentrations in lower trophic level organisms, such as zooplankton and fish (Fisk et al. 2001a) because they cannot form, or slowly form, oxychlordane. Concentrations of oxychlordane in the turbot were low, resulting in very high ratios when compared to studied Greenland shark concentrations (Table 4). Concentrations of oxychlordane in black dogfish collected in Davis Strait were low and  $\sim 0.10$  of trans-nonachlor concentrations (Berg et al. 1997). The ratio of oxychlordane to trans-nonachlor in the Greenland sharks of Cumberland Sound and Davis Strait were 1:2 and 1:16, respectively. One potential source of oxychlordane is ringed seals, and other marine mammals, which have relatively high burdens of oxychlordane (Norstrom and Muir 1994). Oxychlordane concentrations in the studied sharks were weakly correlated (P < 0.1) to  $\Sigma$ chlordanes concentrations, but they were significantly correlated when one outlier was removed (P < 0.05; Fig. 5). Similar results were observed for  $\Sigma$ DDT and  $\Sigma$ PCB. If we assume that Greenland sharks cannot form oxychlordane, individual sharks feeding on seals should have higher levels of oxychlordane. Since OC concentrations in seals are greater than turbot (Table 3), Greenland shark feeding on seals would also be expected to have greater concentrations of OCs compared with sharks not feeding on seals. Therefore, it would appear that the small Davis Strait Greenland sharks are not feeding significantly on seals based on

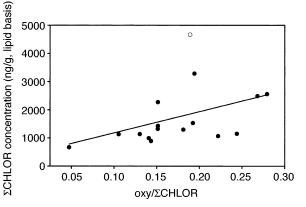


FIG. 5. Relationships between the percentage of oxychlordane in  $\Sigma$ chlordane and  $\Sigma$ CHLOR concentrations (lipid basis, ng/g) in Greenland sharks (closed circles only,  $\Sigma$ -CHLOR = 351 + 6732(oxy/ $\Sigma$ CHLOR),  $r^2 = 0.31$ , P = 0.03). The open circle represents an outlier Greenland shark that was not used in the regression.

relatively low levels of oxychlordane, but that a certain percentage of the larger Cumberland Sound sharks had fed on seals based on higher levels of oxychlordane. The lack of relationships between OC concentrations and length or  $\delta^{15}N$  provides further support that seal consumption is variable in the Greenland sharks studied.

# ACKNOWLEDGMENTS

This work would not have been possible without the intellectual, financial, and spiritual support of Margaret Treble (Freshwater Institute, Winnipeg, Manitoba, Canada). The authors are indebted to George Benz (Southeast Aquatic Research Institute and Tennessee Aquarium, Chattanooga, Tennessee, USA) for a through review of a previous manuscript but more importantly for enlightening conversations about the Greenland shark. We are indebted to the turbot fishermen of Pangnirtung, Nunavut, Canada, particularly Davidee and Adam Evic, Jason Duffe for statistical advice, and for the skillful assistance of Pierre (Pete) Landry.

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