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Stock separation of narwhal (*Monodon monoceros*) in Canada based on organochlorine contaminants.

Discrimination des stocks de narval (*Monodon monoceros*) au Canada en fonction de leurs teneurs en contaminants organochlorés.

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ABSTRACT

This document describes organochlorine contaminants (OCs) in narwhal tissue, analysed for the purpose of stock discrimination, with data available in August 2003.

Canonical discriminant function analysis using 14 OC groups separated narwhals hunted in Repulse Bay, Broughton Island, Pond Inlet and Grise Fiord. Canonical functions were most strongly correlated with the concentrations of several PCB congeners and DDT compounds. While narwhals from all sample locations had overlapping OC contaminant concentrations, OC ratios differed. Repulse Bay narwhals were the most distinct, with overall lower OC levels and high PCB/DDT ratios. Narwhals from Broughton Island had relatively high OC levels and high PCB/DDT ratios. Narwhals hunted in Pangnirtung may be from the same stock as those hunted in Broughton Island. Narwhals from Clyde River were not convincingly associated with or separated from other groups; however this may be due to the small sample size of six animals. Among the 4 major sample groups, narwhals from Pond Inlet and Grise Fiord were the most similar. However, narwhals from Pond Inlet had a notably lower PCB/DDT ratio than those from Grise Fiord. These differences are assumed to be due to food web differences. Several hypothesized stock differences, existing scientific knowledge, and traditional knowledge are confirmed by the results presented here.

RÉSUMÉ

Ce document présente une analyse des contaminants organochlorés (CO) présents dans les narvals. Cette analyse vise à distinguer les stocks et elle est fondée sur les données qui étaient disponibles en août 2003.

Une analyse discriminante canonique de 14 groupes de CO a permis de distinguer les narvals capturés près de Repulse Bay, de Broughton Island, de Pond Inlet et de Grise Fjord. Les fonctions canoniques sont le plus fortement corrélées aux concentrations de plusieurs congénères de BPC et de DDT. Les narvals de tous les lieux d'étude possèdent des teneurs en CO qui se chevauchent, mais les rapports entre ces contaminants diffèrent. Les narvals de Repulse Bay se distinguent le plus de ceux des autres régions puisque, en général, leur teneur en CO est plus basse et leur rapport BPC/DDT est élevé. Les narvals de Broughton Island ont une teneur en CO et un rapport BPC/DDT plutôt élevés. Les narvals de Pangnirtung pourraient appartenir au même stock que ceux capturés près de Broughton Island. Nous n'avons pu établir une distinction ou une ressemblance claire entre les narvals de Clyde River et ceux d'autres groupes; cela pourrait être dû à la petite taille de l'échantillon (six narvals). Parmi les quatre principaux groupes d'échantillons, les narvals

de Pond Inlet et de Grise Fjord se ressemblent le plus. Le rapport BPC/DDT des narvals de Pond Inlet est cependant nettement inférieur à celui des narvals de Grise Fjord. Ces différences pourraient être dues à des variations sur le plan du réseau trophique. Ces résultats confirment plusieurs hypothèses sur les différences entre les stocks ainsi que des connaissances traditionnelles et scientifiques existantes.

Introduction

The narwhal *(Monodon monoceros)* is an ice-associated cetacean that inhabits Arctic seas bordering the Atlantic Ocean. It is the northernmost cetacean common between 70° and 80° N, and occurs less often south to 65° and north to 85° (Reeves and Tracey 1980). The North Atlantic Marine Mammals Commission recognizes 17 aggregations worldwide, which may be discrete or a mixture of stocks (NAMMCO 2000). In the summer months, narwhals visit inshore bays and fiords in the Canadian archipelago, Greenland, and Foxe Basin (Fig. 1). The International Whaling Commission currently recognizes two stocks of narwhals in the Canadian Nearctic. One is centered in northern Hudson Bay and southern Foxe Basin in the summer, and the other in the fiord waters of Northwest Greenland and the Canadian High Arctic archipelago (IWC 1999). IWC also suggests that a working hypothesis in which discrete stocks occupy separate summering areas in the open water season.

In the autumn when fast ice forms, high Arctic stocks of narwhals move from summering areas and spend the winter in areas covered by dense offshore pack ice (Dietz and Heide-Jørgensen 1995). During winter months, narwhals are widely dispersed in Baffin Bay and Davis Strait with high concentrations between 55°-64° W and 68°-71° N and off Disko Bay (Koski and Davis 1994, Heide-Jørgensen *et al.* 1993) (Fig. 1). In spring, narwhals are seen along ice edges on the east coast of Baffin Island, at the entrance of Lancaster Sound and Jones Sound, and in Smith Sound (Bradstreet *et al.* 1982, Koski and Davis 1994). Narwhals from this stock may reach northern Foxe Basin by Fury and Hecla Strait (Stewart *et al.* 1995). Narwhals are also known to move along the ice edges in West Greenland and to concentrate at the entrance of Inglefield Bredning (Born *et al*. 1994).

In Canada, the harvest in the high Arctic takes place from Grise Fiord, Pond Inlet, Arctic Bay, Resolute Bay, Creswell Bay, Taloyoak, Kuugaruk, Broughton Island, Pangnirtung, Clyde River, and several locations in Foxe Basin, namely Igloolik, Hall Beach, and Repulse Bay (Fig. 1) (DFO 1998a). In West Greenland, the hunt takes place in five areas: Qaanaaq, Upernavik, Uummannaq, Disko Bay, Sisimiut, and in the south (Fig. 1).

Heide-Jørgensen e*t al.* (2001) proposed a model of the dispersal of narwhals in Baffin Bay and adjacent area based on satellite tracking, genetic studies, and compilations of local knowledge. Nine coastal summering concentrations of narwhals, proposed to constitute stocks, are identified. Also, nine major hunting grounds in Canada and Greenland are identified and several stocks are hunted on several hunting grounds. Narwhals hunted in Grise Fiord are hypothesized to be part of a "Jones Sound" or possibly a "Smith Sound" stock or aggregation. These narwhals may also be hunted in Qaanaaq, Greenland. Heide-Jørgensen *et al.* (2001) believed that whales hunted in other communities in Canada are most likely not hunted in West Greenland. According to the Heide-Jørgensen *et al.* (2001) model, the narwhals hunted in both Broughton Island and in Clyde River are described as "East Baffin small stocks". Whales hunted in Pond Inlet are presumed to be part of an "Eclipse Sound Aggregation". Arctic Bay hunts from the "Admiralty Bay" aggregation, but possibly from the "Eclipse Sound" or "Somerset" aggregations. Narwhals hunted in Creswell Bay and from Resolute are most likely part of "Somerset" aggregation, and it is possible that Arctic Bay also hunts these narwhals.

Two of three hunters from Grise Fiord believed narwhals in Jones Sound to be a different stock from those in the Pond Inlet/Arctic Bay area because of behavioral differences (Stewart *et al*. 1995). Narwhals in the Grise Fiord area are more readily herded into shallow water than those off Lancaster Sound and off Greenland. Visitors from Greenland and Pond Inlet have remarked on how, compared to other areas, narwhals at the floe edge in Jones sound are not alarmed at the sight of hunters. However, many narwhals that frequent the Grise Fiord area are believed to be young (Stewart *et al*. 1995). Hunters in Arctic Bay did not describe different groups of narwhals apart from males and females.

The summer range of northern Hudson Bay narwhals includes the waters surrounding Southhampton Island, with the largest aggregations in Repulse Bay, Frozen Strait, Western Foxe Channel, and Lyon Inlet (Richard 1991; DFO 1998b) (Fig. 1). These narwhals are assumed to winter in eastern Hudson Strait or in open leads and polynas of northern Hudson Bay and western Hudson Strait. They are assumed to be a separate stock because of their apparent year-round discontinuous distribution with Baffin Bay narwhals. Unlike the hunters from Arctic Bay and Grise Fiord, no hunters from Repulse Bay had killed a tusked narwhal and found it to be female (Stewart *et al.* 1995). Hunters in Igloolik did not describe different groups of narwhal.

To date, DNA evidence has confirmed the existence of different stocks of narwhals in Canada and Greenland (de March *et al.* 2003, Palsbøll *et al.* 1996). However, in spite of statistically significant differences, there is a very high degree of overlap of genetic characteristics among all populations that have been examined. Thus we consider molecular genetics to be a weak tool for identifying stocks.

Organochlorine (OC) contaminants have been used to determine stock affiliations of whales in univariate and multivariate analyses (Aguilar 1987, Aguilar *et al.* 1993; Stern *et al,* 1994, Innes *et al.* 2002, Krahn *et al.* 1999, de March *et al.* 1998). Different patterns of OC concentrations in marine mammals are caused by differences in feeding, which may depend on different prey species, trophic status of prey species, and feeding patterns in summering areas, wintering areas, on migration routes, and possibly on the feeding behaviour of different social groups. Analysis of eastern North American white whale *(Delphinapterus leucas)* using OC data from our laboratory showed that there were overall differences in OC concentrations among beluga samples from Greenland, Grise Fiord, Pangnirtung, Iqaluit, Kimmirut, and several Hudson Bay locations (Innes *et al.* 2002, de March *et al.* in press). The strongest separation was between belugas that fed in Hudson Bay as opposed to Atlantic waters (de March *et al.* in press.). The pattern of separation among belugas was supported by genetic data except for the lack of demonstrated genetic difference between belugas from Grise Fiord and West Greenland (de March et al. 2002).

Methods

This study examines and compares OC concentrations in 125 narwhals hunted in the communities of Broughton Island, Grise Fiord, Pond Inlet, Clyde River, Pangnirtung, and Repulse Bay between 1993 and 2001 (Table 1). Blubber samples were frozen and stored at -20°C until organochlorine contaminant (OC) analyses were done. Both lengths and sex were available for 102 narwhals.

Determinations of organochlorine contaminants in blubber tissues followed the procedures described by Stern *et al.* (1994). Blubber samples were partially thawed and 2 g were combined with anhydrous Na_2SO_4 (heated at 600°C for 6 hours prior to use). The mixture was then extracted twice with hexane in a small (50ml) ball mill, with the hexane decanted between extractions. Surrogate recovery standards of PCB30 and octachloronaphthalene (OCN) were added prior to extraction. Extractable lipids were determined gravimetrically on a fraction (1/10) of the extract. A portion of the extract equivalent to approximately 100mg lipid was separated into three fractions of increasing polarity on Florisil (8g; 1.2 % v/w water deactivated). The first fraction was eluted with hexane and contained PCBs, DDE, *trans*-nonachlor, and mirex; the second fraction was eluted with hexane:DCM (85:15) and contained HCHs, most chlorinated bornanes, chlordanes and most DDTs. Some chlorobornanes, most notably T2 (Parlar no. 26), were partially eluted with hexane. The third fraction, containing dieldrin and heptachlor epoxide, was eluted with a 1:1 mixture of hexane:DCM. After addition of aldrin as a volume corrector, each fraction was analyzed for OCs by capillary gas chromatography (GC) with ^{63}Ni electron capture detection (ECD) by means of an automated Varian 3400 GC (Varian Instruments, Palo Alto, CA). Samples were injected on a 60m x 0.25mm i.d. DB-5 column (film thickness = 0.25um). H_2 was used as the carrier gas (2 mL/min) and N_2 as the make-up gas (40mL/min). A total of 103 PCB congeners (including co-eluting congeners) and 40 OC pesticides were quantified using external standard mixtures (Ultra Scientific, North Kingstown, RI).

Recoveries of the surrogates, PCB-30 and OCN were uniformly greater than 90% and no corrections were made for recoveries. Other quality assurance measures included the analysis of standard reference materials (NIST cod liver oil 1588) and duplicated analysis of every 12th sample. Samples from all locations and years were extracted and analyzed in random batches so that observed differences could not be attributed to any systematic analytical variation. The FWI laboratory participates in numerous inter-laboratory comparisons (e.g., IAEA-MEL (International Atomic Energy agency-Marine Environmental Laboratory), ICES PCB analysis program and the NCP quality assurance program for organochlorines.

Laboratory analyses were performed using the same methodology, instrumentation and analyst over a period of six years. Duplicate results were satisfactory, and results were averaged for the duplicated analyses.

One hundred and thirty-five (135) organochlorine (OC) compounds, some co-eluting, were quantified. One hundred and five (105) OC compounds had consistent non-zero values and these compounds were kept for statistical analyses. Sums of concentrations for 17 OC groups (Table 2) were also calculated. Data were examined as *ng/g* or *ppb* wet blubber weight and as lipid corrected values. To test for the significance of the covariates sex and length, the model:

$$\log(concentration) = a_s \times sex + b \times length + c_s \times sex \times length + d \times location, \qquad (1)$$

was examined for all OC and OC groups using the general linear models procedure (PROC GLM) in SAS (1989). The terms '*sex'* (= M or F) and '*location'* (6 locations) are class variables; *'length',* a continuous variable; a_s, the sex effect; d_l, the location effect; *b*, the coefficient describing the effect of *age*; and c_s, coefficient of the *sex* × *length* effect for each sex. The coefficients b and c_s were significant at a very low frequency, thus the model was reduced to:

$$\log(concentration) = e_s \times sex + f \times location, \qquad (2),$$

in which e_s is the sex effect. Raw logged values for each OC and OC group were then corrected for sex with coefficients from Equation (2) as follows:

$$\log(concentration)_{adjusted} = \log(concentration)_{observed} - e_s \times sex, \qquad (3),$$

in which the sex was assumed to be male. Because of the covariate correction, results of further analyses are not expected to correlate with sex.

The significance of the sex effects, location effects, as well as the values and partial probabilities (Type III error) of contrasts comparing location effects for each OC and OC group were also calculated for individuals (Option CONTRAST in PROC GLM in SAS 1989) (Table 2).

OC concentration patterns among sampling locations were described using Canonical Discriminant Analysis (CDA) with the PROC DISCRIM and PROC STEPDISC procedures (SAS 1989). The probabilities of population memberships were obtained by 'crossvalidating' all individuals (Option CROSSLIST in PROC DISCRIM in SAS 1989) and groups of individuals (Option TESTCROSS in PROC DISCRIM in SAS 1989). In crossvalidation, the individual or group to be tested is removed from the data, the canonical functions are calculated without the individual(s), and then the individual or group placed with the functions from the reduced data set (Lachenbruch and Mickey 1968).

In view of concerns about overparameterization and lack of power caused by too many predictor variables (Tabachnick and Fidell 2000; de March *et al.* in press, also see Discussion), we performed the CDA with a limited number of predictor variables. The nineteen predictor variables tested first were : ∑DDT (o,p'- and p,p'-DDT), ∑DDE, ∑DDD, ∑HCH, ∑CHL, ∑TOX, ∑HCB, ∑3-CBs (trichlorobiphenyls), ∑4-CBs (tetrachlorobiphenyls), ∑5-CBs (penta-…), ∑6-CBs, ∑7-CBs, ∑8-

CBs, ∑9-CBs, ∑1&2-CBs, endrin, mirex, endosulfan, and octachlorostyrene (Table 2). Samples from Pangnirtung and Clyde River were omitted from the first analysis because it was believed that their small sample size might cause overparameterization. The number of OCs and OC groups used in the CDA was then reduced based on their correlations with the canonical functions. Samples from Clyde River and Pangnirtung were included in subsequent analyses.

The clustering algorithm for the figure accompanying Table 3 was available in the PHYLIP v 3.5 package (Felsenstein 1993).

Results

Assuming the regression coefficient describing effects of sex is "0" for males for all OCs and OC groups, the coefficient for females ranged from -0.448 for PCB158 to -0.0698. The mean value of the coefficient for females was -0.253 ± 0.069313 (Mean ± SD, n=122 (105 OCs & 17 OC groups)). Sex was a significant covariate ($p < 0.05$) in all 17 OC groups and in 80/105 individual OCs (Table 2).

There were significant location effects in 14/17 OC groups and in 80/105 OCs (Table 2). Repulse Bay narwhals had significantly different OC concentrations from the five other locations in 10/17 OC groups. Narwhals from Baffin Island communities differed from other high Arctic communities in 3/17 OC groups, and concentrations in Pond Inlet and Grise Fiord differed in 9/17 OC groups. Some locations also differed in concentrations of octachlorostyrene, mirex, endrin, and dieldrin (Table 2).

Five of 19 predictor variables were dropped after the initial CDA (without Pangnirtung and Clyde River samples) because they were not strongly correlated with canonical scores ($p < 0.01$). Dropped predictor variables were diedrin, endrin, mirex, ∑5-CBs, and ∑8-CBs, leaving the 14 variables ∑DDT, ∑DDE, ∑DDD, ∑HCH, ∑CHL, ∑TOX, ∑HCB, ∑3-CBs, ∑4-CBs, ∑6-CBs, ∑7-CBs, ∑9-CBs, ∑1&2-CBs and octachlorostyrene. Three of the dropped variables did have significant location effects, but were dropped because the CDA showed that they were redundant and thus caused concerns about overparameterization.

The first canonical score accounted for 51.9% of the variation; the second, 25.1%; the third, 15.6% (p likelihood ratios < 0.0001); and the remaining two scores 7.4% (p likelihood ratios > 0.15) . The first score was strongly correlated with ∑4-CBs (r = 0.456), ∑DDD (0.453), ∑6-CBs (0.383), ∑9-CBs (-0.359), ∑PCBs (sum of all congeners)((0.343), all at p <0.0001, and with dieldrin, ∑HCB, ∑HCH, ∑5-CBs, ∑7-CBs, and ∑DDE at $p \leq 0.05$. This score primarily separated the four major samples in the order Repulse Bay, Pond Inlet, Grise Fiord, and then Broughton Island (Fig. 2). Individual narwhal and groups of narwhals that had high scores for this function were crossvalidated as being from Broughton Island (Table 3). Notably, 3/7 narwhals from Pangnirtung were cross-validated to Broughton Island.

The second canonical score was strongly correlated with ∑DDE (r = 0.558), ∑3-CBs (0.469) , ∑1&2-CBs (0.444), octachlorostyren*e* (r=0.404), ∑HCH (0.371), ∑DDT (0.369), ∑CHL (0.335), and ∑DD (∑DDE + ∑DDD + ∑DDE) (0.489), all at p <0.0001, and with dieldrin, mirex, endrin, ∑HCB, ∑TOX, ∑4-CBs, ∑7-CBs, ∑8-CBs, ∑9-CBs and ∑DDD at $p \leq 0.05$. Repulse Bay narwhals scored lowest, and Pond Inlet narwhals scored highest. (Fig. 2) . Other locations had intermediate scores. In the crossvalidations, 12/18 narwhals from Repulse Bay were correctly crossvalidated. Sixteen/25 narwhals from Pond Inlet were correctly crossvalidated, but 5/25 of these narwhals were crossvalidated to Grise Fiord (Table 3). Pond Inlet narwhals had the highest mean OC concentrations, while Repulse Bay narwhals relatively low concentrations. The lowest mean concentrations of OCs were observed in Clyde River narwhal, but this range was within OC ranges observed in other locations.

The third canonical score was correlated with ∑1&2-CBs (r = -0.247, *p*=0.005)), octachlorostyrene (-0.243, *p*=0.006) , endrin (-0.233, *p*=0.009), and ∑TOX (r = 0.185, *p*=0.049). This score mainly separated narwhals from Grise Fiord from other locations. Narwhals from Grise Fiord had lower levels of ∑1&2-CBs and octachlorostyrene, and higher levels of ∑TOX than other locations. Thirteen/18 narwhals from Grise Fiord were correctly crossvalidated, while 5 were placed to various communities (Table 3).

Figure 3 shows the levels of ∑4-CBs, ∑DDE, and ∑1&2-CBs, the three OC groups most correlated with the three significant canonical scores, plotted against each other. It should be noted that many different OCs could have been chosen for these plots, and the degree of separation among locations would have been similar.

The results of stepwise CDA using all 17 OC groups were also examined to assist in interpreting results. The first four entered OCs or OC groups were ∑DDE (R^2=0.067), then mirex (cumulative R^2=0.177), ∑6-CBs (cum R^2=0.308) and then ∑9-CBs (cum R^2=0.461).

Most individuals from major locations correctly crossvalidated (First section, Table 3). Percentages are: Broughton Island 33/51=65%, Grise Fiord 13/18=72%, Pond Inlet 16/25=64%, and Repulse Bay 12/18=67%. There appears to be no pattern to the misclassifications. Narwhals groups are crossvalidated less convincingly than individuals are (Second section, Table 3). Narwhal from Broughton Island and Grise Fiord are the only ones convincingly crossvalidated to their source.

The pairwise generalized squared distances (Fisher 1940) between the six locations shown in Table 4 are related to misclassification rates. In the tree based on neighbor-joining, the distance between locations is approximately equal to the squared distance (figure with Table 4).

Figure 3 shows a bivariate plot of ∑7-CBs and ∑DDD, the two OC groups that demonstrated overall differences in the analysis of beluga OC data (de March *et al.* in press).

Discussion

Several hypothesized stock differences are confirmed by the results presented here. It is highly probable that narwhals from Repulse Bay, Grise Fiord, Pond Inlet, and from east Baffin Island represent three different stocks. The results of this OC study confirm the results of the traditional knowledge study of Stewart *et al.* (1995).

The narwhals hunted in Repulse Bay stand out in the CDA analysis, and are separated out by two of the three significant canonical functions. Narwhals from Repulse Bay have lower overall OC levels and slightly different OC ratios than the high Arctic samples. The Repulse Bay narwhals have long considered to be a separate stock because of their geographic separation from other sample groups.

Narwhals sampled at Broughton Island appear to be a stock distinct from Grise Fiord, Pond Inlet, and Repulse Bay. The OC profiles of the Broughton Island narwhal appear to be consistent from year to year. Also, the narwhals hunted in Cumberland Sound by hunters from Pangnirtung may be the same stock as Broughton Island narwhals since they have have very similar OC profiles. The close geographic proximity of these two groups also makes this plausible. Narwhals from Clyde River are not distinct in these analyses, showing some similarities to all high Arctic groups tested, but mostly to Pangnirtung and Broughton Island. A larger sample size is needed to describe narwhals from Clyde River.

Narwhals from Pond Inlet and those from Grise Fiord may represent different but overlapping stocks. It is known that narwhals from Pond Inlet migrate past Grise Fiord, so the narwhals from Pond Inlet might be harvested in Grise Fiord. The two groups' OC profiles do overlap, and this observation is not inconsistent with our knowledge of migrations. It is possible that more than one stock is hunted in Grise Fiord, or that some narwhals from Pond Inlet do not migrate past Grise Fiord. Narwhals from Pond Inlet are the hypothesized “Eclipse Sound” stock, while narwhals from Grise Fiord are the hypothesized “Jones Sound” stock (Heide-Jørgensen e*t al.* 2001). It would be of interest to sample narwhals from Greenland to see if their OC profiles are similar to those from Grise Fiord.

The OCs that demonstrated strong location effects (Table 2) were not necessarily the same ones that contributed most in the multivariate CDA analysis. This is because the CDA analysis searches for linear combinations that optimize differences among the locations being examined. Since data are log-transformed, separation of sample groups by CDA can be caused not only by differences in OC levels, but also by differences in OC ratios. Some of these ratios are demonstrated in Figures 2 and 3. These are not the only OCs that have could been plotted here, but they are representative of differences demonstrated by the three canonical scores. The results of the stepwise CDA, in which three different OCs and OC groups are chosen first, again demonstrates that differences cannot be attributed to only a small number of OCs.

All differences are assumed to be due to differences in feeding patterns, food web position, different water masses, and to different OC transportation rates to the Arctic. Many of the details of these differences, particularly those related to feeding and food webs, are only speculation at this point. Higher average OC levels are generally assumed to indicate a higher level in a food web. In this study, narwhals from Broughton Island and Pond Inlet have the highest levels overall, and those from Repulse Bay the lowest.

In the study of de March *et al*. (in press), ∑7-CBs and ∑DDD were the most important OC groups that separated belugas hunted in Kimmirut and Iqaluit from those hunted in Pangnirtung. In this study, both of these OC groups were weakly but significantly correlated with both canonical scores 1 and 2. The absolute value of “r” correlating ∑7-CBs and ∑DDD with these scores ranged from 0.215 to 0.339, all significant at $p < 0.03$. As also observed in belugas, high Arctic narwhals had a lower ∑PCB/∑DDD ratio than those summering in Hudson Bay (Fig. 3).

It is difficult to compare OC ratios between narwhals and beluga because covariate corrections were different in the two studies and because the study with beluga did not use lipid-corrected data. Nevertheless, given an assummed correction of between 80-90 % lipid in beluga muktuk, the lowest OC levels in both species were observed in Repulse Bay narwhals and Cumberland Sound beluga. The highest levels were observed in narwhals from Broughton Island and beluga from Iqaluit.

In an OC stock discrimination study with beluga (de March *et al*. in press), both whale “age” and “sex” were significant covariates. In this study with narwhal, only “sex” was consistently significant while “length” was not. This may be because the length measurement may not be a good indicator of whale age. Also, the size of narwhals that were hunted may have been within a small range. As in the beluga, the significant “sex” covariate confirms that females accumulated fewer OCs than males.

As for all results analysed with statistical methodologies, two sample populations that are “significantly different” are not completely different. Statistical significance at $p = 0.05$ means that the degree of difference observed would be entirely due to chance in 5% of cases if the two populations really are one population. If two populations have different average results, the probability of demonstrating “significant differences” increases with sample size. Two sample groups may also be “significantly different” if the compared samples, sampled from the same larger populations, have not been randomlyampled. For example, a group of relatives may have been sampled. Thus, two large sample groups can have a very high degree of overlap of OC profiles and still be significantly different. Our primary interest should be in the degree of overlap, and our second in the confidence that samples populations are different.

Another possible complication is that if sampled groups are small, significant differences can be due to chance.

In this study, all sample populations have overlapping OC concentrations. We cannot know whether these are separate stocks that have overlapping concentrations, or whether different stocks are mixing in different areas. This can only be understood by monitoring all individuals in all populations.

Although we have tried to avoid methods that create statistical artifacts, the possibility of artifacts must always be kept in mind. Canonical discrimation analysis finds OCs that discriminate defined sample groups. Thus, if there are only 5 samples from a location and all 5 have high concentrations of a particular OC entirely due to chance, this location will stand out as being distinctly different. The probabability of this happening is very high if many OCs are used in the statistical analyses. Therefore in this study, the number of OCs used in the analyses was reduced based on their performance in analyses using only locations with larger sample sizes. In this study, both Pangnirtung and Clyde River were initially excluded because of their small sample size. When they were included in analyses with reduced predictor variables, their ordination seems reasonable in view of what we know about these groups.

It has often been suggested that methods that cluster whales without *a priori* consideration of sample location would be a more meaningful method for discriminating stocks. However, even these methods require that OCs involved are chosen ahead of time. As descibed previously, there many be many OCs to choose from, and choosing these may be a problem. Another complication is the fact that such methods seldom take into consideration that OC profiles between stocks should overlap if there is a normal distribution of values within each stock. Nevertheless, there is no doubt that such methods will receive more attention in the future.

A copy of a SAS program which includes all described analyses is available from the first author.

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Table 1. Sampling locations, sample sizes, sex ratio, and narwhal lengths.

	n narwhal	n by year	F : M	Length (cm) Mean	SD	n	Range
Broughton Island	51	9 1993, 29 1995, 13 1996	18 : 32	426	55	51	260-529
Pangnirtung	7	7 1996	1 : 5	379	66	7	305-489
Clyde River	6	6 1995	4 : 2	389	31	6	347-426
Grise Fiord	18	1 1993, 5 1995,1 1996, 11 1999	2 : 12	405	81	15	268-567
Pond Inlet	25	6 1992, 9 1994, 10 1999	7 : 8	384	47	14	286-454
Repulse Bay	18	4 1993, 14 2001	8 : 10	409	43	13	315-488
Total	125		40 : 69				

Table 2. Statistics for selected organochlorine contaminants (OCs) for narwhal. Means and CVs of lipid- and sex-corrected concentrations in *n*g/g (ppb) are given for six hunting locations. Other probabilities were obtained from statistics associated with Equation (2), and include significance of covariates, of overall location effects ($p \leq 0.05$ indicated with a *), and of three contrasts comparing OC concentrations among six locations. "RB *vs* 5 others" is the significance of Repulse Bay differing from the other 5 locations, "BA *vs* HA", the significance of the Baffin locations vs Grise Fiord and Pond Inlet, and "PI vs GF", the significance of Grise Fiord vs Pond Inlet.

	Repulse Bay n=18		Broughton Island n=51		Pangnirtung n=7		Clyde River n=6		Grise Fiord n=18		Pond Inlet n=25						
OCs and OC Groups	Mean ng/g	CV (%)	Mean ng/g	CV (%)	Mean ng/g	CV (%)	Mean ng/g	CV (%)	Mean ng/g	CV (%)	Mean ng/g	CV (%)	p Sex Differences	p Location Differences	RB vs 5 others	BA vs HA	PI vs GF
ΣCHL	1502	54	1736	35	1907	14	1070	12	1640	26	2075	51	0.0045 *	0.0016 *	0.0346	0.1308	0.0031
ΣCBz	376	57	519	38	484	12	264	16	399	32	519	66	0.0077 *	0.0032 *	0.0524	0.7909	0.0267
ΣTOX	6792	50	6923	56	9114	36	5601	31	8703	22	8661	62	3.8E-04 *	0.2536	0.2395	0.2091	0.6496
ΣHCH	101	67	130	37	127	22	67	19	117	30	140	50	0.0363 *	0.0002 *	0.0094	0.1313	0.0068
Σ1&2-CBs	28	59	35	44	39	21	22	14	32	47	55	79	0.0461 *	1.7E-07 *	0.0027	0.0057	3.5E-06
Σ3-CBs	112	60	135	33	124	12	83	17	151	27	153	35	0.0108 *	0.0013 *	0.0076	0.0132	0.1283
Σ4-CBs	439	59	731	40	629	20	328	15	544	24	623	49	0.0063 *	3.5E-05 *	0.0041	0.7302	0.0851
Σ5-CBs	1044	47	1311	39	1405	22	743	21	1092	26	1327	59	7.0E-04 *	0.0140 *	0.1817	0.7019	0.0147
Σ6-CBs	1312	52	1883	40	1934	18	1008	20	1419	25	1678	56	0.0019 *	0.0029 *	0.0368	0.6528	0.0580
Σ7-CBs	433	51	584	40	597	21	326	21	483	20	556	52	6.5E-04 *	0.0148 *	0.0644	0.9353	0.1056
Σ8-CBs	74	58	77	43	88	21	55	25	75	29	83	45	0.0012 *	0.6342	0.4404	0.7512	0.1982
Σ9-CBs	4.2	83	2.5	74	3.4	51	1.9	39	3.3	30	4.4	64	3.1E-04 *	0.0110 *	0.1210	0.1089	0.2167
ΣPCBs	3575	50	4887	38	4949	19	2690	18	3923	23	4601	52	0.0014 *	0.0034 *	0.0472	0.9235	0.0354
ΣDDT	1231	62	1190	46	1259	29	788	22	1388	33	1556	41	0.0039 *	0.0813	0.2416	0.0473	0.1110
ΣDDE	1761	70	2786	54	3285	40	1677	32	2468	47	3635	51	0.0090 *	2.7E-06 *	1.5E-05	0.1114	0.0013
ΣDDD	362	54	645	46	544	32	306	37	461	25	550	59	0.0078 *	2.3E-05 *	0.0026	0.6106	0.0150
ΣDDs	3363	63	4630	48	5098	34	2790	25	4323	37	5762	47	0.0058 *	9.9E-05 *	5.9E-04	0.0906	0.0038
octachlorostyrene	296	45	287	35	318	20	194	19	269	28	414	51	0.0012 *	1.2E-04 *	0.8679	0.0296	1.79E-05
mirex	23	78	18	42	18	32	11	25	21	25	21	56	8.9E-04 *	0.0067 *	0.4514	0.0024	0.0238
endrin	23	53	19	46	26	8	14	11	18	51	22	52	0.0750	0.0177 *	0.7107	0.5535	0.0058
dieldrin	245	64	309	48	329	34	178	32	298	25	299	64	1.4E-04 *	0.1409	0.0374	0.7232	0.9543
Selected OCs with strong location differences																	
PCB26 (3 Cl)	5.3	73	13	47	13	45	6.0	47	8.0	34	7.3	41	0.0946	4.3E-08 *	3.3E-06	0.2760	0.3088
PCB31 (3 Cl)	41	55	49	34	42	19	30	25	56	27	69	46	0.0159 *	7.3E-06 *	0.0113	1.4E-04	0.0046
PCB66 (4 Cl)	13	82	87	59	48	76	18	70	21	53	20	38	0.6087	3.3E-12 *	1.8E-05	3.0E-02	0.9353
PCB58 (4 Cl)	20	54	24	46	28	21	16	12	24	46	44	90	0.0579	5.4E-09 *	0.0035	4.9E-04	6.3E-07
PCB149 (6 Cl)	157	62	344	48	305	40	141	47	175	20	245	85	0.0238 *	4.6E-06 *	0.0052	0.2513	0.0330
PCB179 (7 Cl)	10	86	21	50	17	33	9	45	14	21	15	58	0.0161 *	6.1E-07 *	6.1E-06	0.5903	0.2768
PCB198 (8 Cl)	2.7	71	2.2	62	3.2	32	1.8	22	2.6	35	5.1	48	0.0180 *	1.6E-05 *	0.1067	0.0174	3.8E-04
p,p'-DDE	1708	70	2729	55	3224	41	1638	33	2412	47	3555	52	0.0088 *	2.6E-06 *	1.2E-05	0.1178	0.0015
p,p'-DDD	299	56	553	48	469	37	264	40	387	26	476	62	0.0096 *	2.2E-05 *	0.0016	0.6740	0.0171
heptachlor epoxide	4.7	88	8.0	55	13	20	4.8	51	5.5	40	4.3	35	0.1586	1.8E-05 *	1.7E-04	0.0041	0.5721
β-hch	38	74	50	45	45	31	22	20	34	32	53	58	0.0582	6.8E-05 *	0.0477	0.2715	4.0E-04
α-hch	45	60	58	34	61	19	34	24	63	31	68	46	0.0220 *	1.2E-04 *	0.0019	0.0299	0.0177
trans-nonachlor	646	65	917	41	933	27	450	16	684	35	945	67	0.0109 *	1.5E-04 *	0.0061	0.4685	0.0025
cis-nonachlor	276	49	224	49	335	18	227	25	352	27	373	33	0.1267	1.5E-04 *	0.3475	0.0174	0.2760

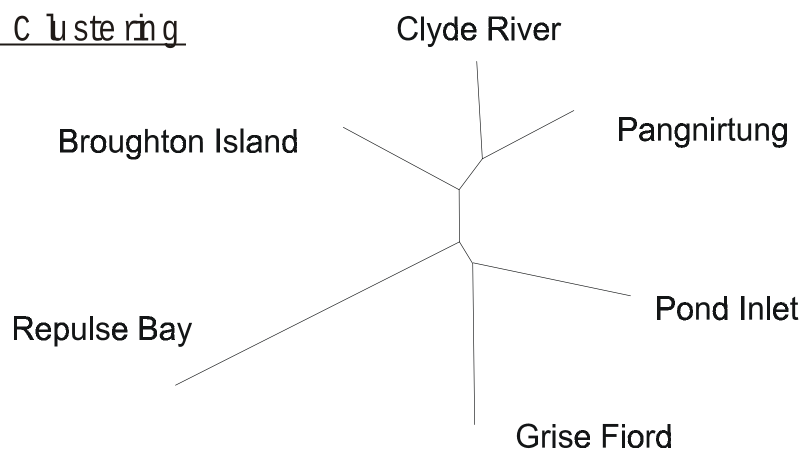
Table 3. Crossvalidation of individual narwhal and yearly collections to the remaining samples. "-" indicates that crossvalidation to a location was not an option because there were no remaining samples for that location.

		Cross-validated to						
		Broughton Island	Pangnir tung	Clyde River	Grise Fiord	Pond Inlet	Repulse Bay	Total
Individual Narwhal from ...	Broughton Island	33	10	4	2	1	1	51
	Pangnirtung	3	2	1	0	1	0	7
	Clyde River	0	2	1	1	1	1	6
	Grise Fiord	0	1	1	13	2	1	18
	Pond Inlet	0	5	1	2	16	1	25
	Repulse Bay	0	1	2	1	2	12	18
								125
Groups of Narwhal from ...	Broughton Island 1993	8	1	0	0	0	0	9
	Broughton Island 1995	22	4	1	2	0	0	29
	BroughtonIsland 1996	3	5	3	0	1	1	13
	Pangnirtung 1996	3	-	2	0	1	1	7
	Clyde River 1995	0	3	-	1	1	1	6
	Grise Fiord 1995 & 1996	0	1	1	1	2	1	6
	Grise Fiord 1999	0	0	2	9	0	0	11
	Pond Inlet 1992	0	3	0	1	1	1	6
	Pond Inlet 1994	0	3	6	0	0	0	9
	Pond Inlet 1999	0	0	2	8	0	0	10
	Repulse 1993	0	1	1	1	0	1	4
	Repulse 2001	0	0	6	0	5	3	14

Table 4. Pairwise generalized squared distances (Fisher 1940) between 6 sample groups. Two methods are used to cluster these distances: a, UPGMA method (Unweighted Pair Group with Arithmetic Mean" method (Sneath and Sokal 1973), and the Neighbor-Joining Method (Saitou and Nei 1987). Resulting trees are presented below the Table.

	Broughton Island	Pangnirtung	Clyde River	Grise Fiord	Pond Inlet	Repulse Bay
Broughton Island	0.00					
Pangnirtung	4.45	0.00				
Clyde River	6.94	4.15	0.00			
Grise Fiord	8.76	9.34	5.59	0.00		
Pond Inlet	9.82	8.16	5.68	6.79	0.00	
Repulse Bay	14.07	9.45	7.67	9.89	10.55	0.00

UPGMA Clustering



Neighbour-Joining Clustering

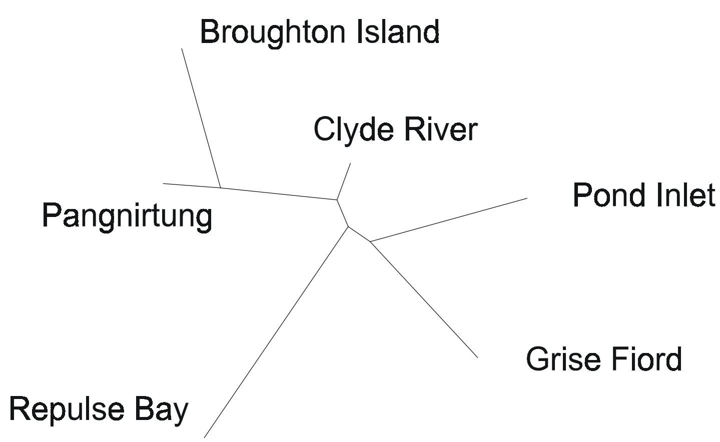


Figure 1. Locations in this manuscript and in de March *et al.* (2003).

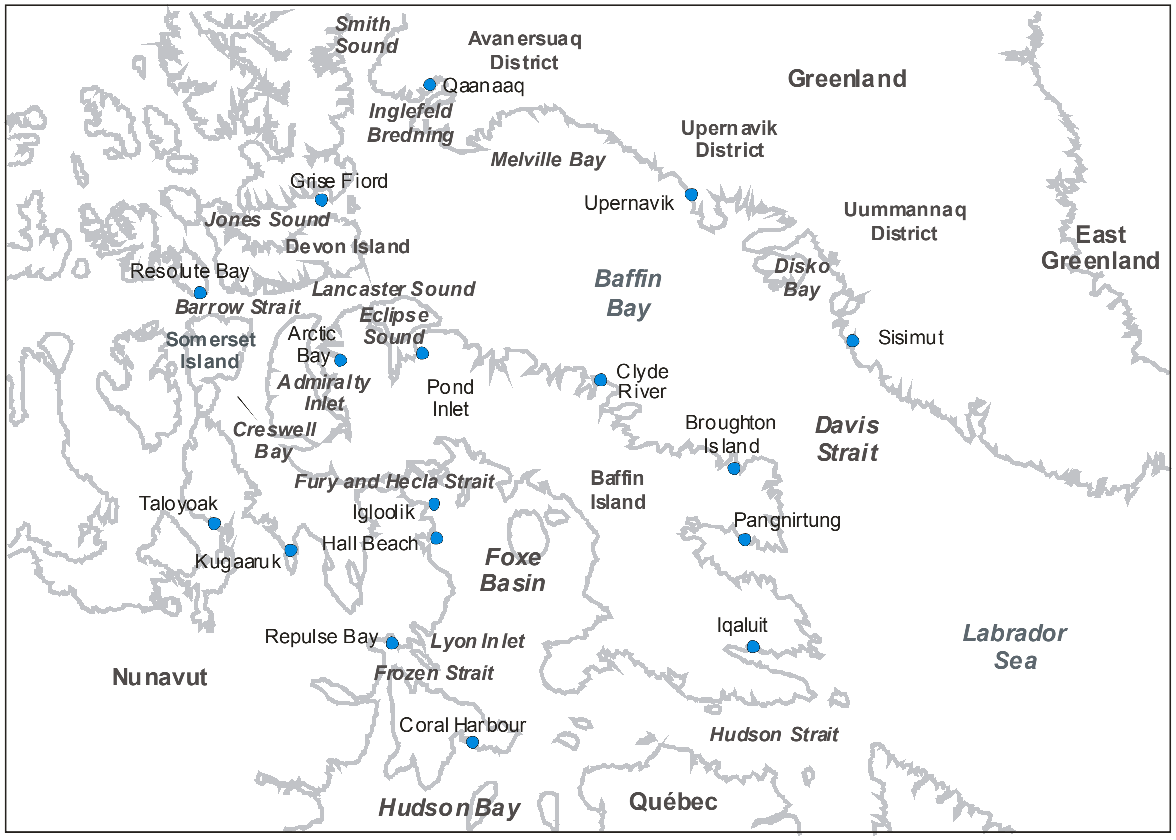


Figure 2. Discrimination of Narwhal Samples with OCs

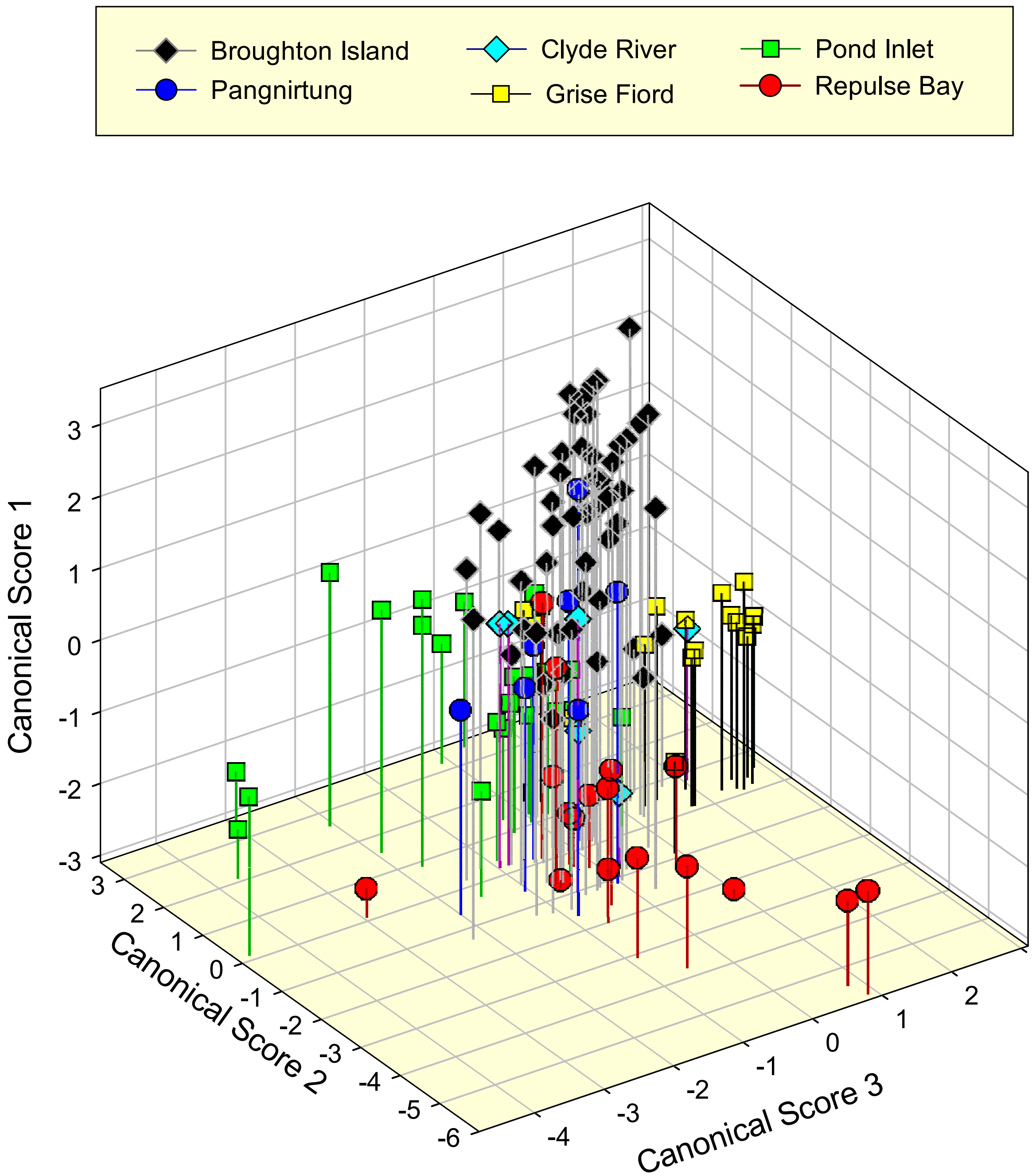


Figure 3. Bivariate plots of selected OC concentrations in 6 locations. Σ4–CBs is correlated with the first canonical score (r=0.46), ΣDDE with the second (r=0.56), and Σ1&2–CBs with the third (r=-0.25).