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Ministry of the Environment

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A multidisciplinary approach

NERI Technical Report No. 400

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Data sheet

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Serial title and no.:	NERI Technical Report No. 400
Publisher:	Ministry of the Environment National Environmental Research Institute ©
URL:	http://www.dmu.dk
Date of publication:	June 2002.
Editing complete:	June 2002.
Referee:	Poul Johansen
Please cite as:	Riget, F., Dietz, R., Møller, P., Glasius, M., Pallsbøl, P. & Hobson, K. 2002. Population structure of West Greenland narwhals. A multidisciplinary approach. – National Environmental Research Institute, Denmark, NERI Technical Report 400.

Abstract: The hypothesis that different populations of narwhals in the West Greenland area exist has been tested by different biomarkers (metal and organochlorine concentrations, stable isotopes and DNA).
Samples of muscle, liver, kidney, blubber and skin tissues of narwhals from West Greenland have been analysed for heavy metals, organochlorines, stable isotopes and DNA. The obtained results of metal concentrations and DNA were included in the existing database, whereas no previous data on organochlorines and stable isotopes in West Greenland narwhals existed.
The metal and POP concentrations and stable isotopes could not support the population structure with two West Greenland populations suggested by the genetic study.

Keywords:	Narwhals, population structure, stable isotopes, haplo-types, heavy metals, organochlorines, West Greenland.
Financial support:	The Danish Environmental Protection Agency as part of the environmental support program Dancea – Danish Cooperation for Environment in the Arctic.
ISBN:	87-7772-678-2
ISSN (electronic):	1600-0048
Number of pages:	53
Circulation:	250
Internet-version:	The report is also available as a PDF-file from NERI's homepage http://www.dmu.dk

Summary

Samples of muscle, liver, kidney, blubber and skin tissues of narwhals from West Greenland have been analysed for heavy metals, organochlorines, stable isotopes and DNA. The obtained results of metal concentrations and DNA suplement excisting data, whereas no previous data on organochlorines and stable isotopes in West Greenland narwhals existed.

The hypothesis that different populations of narwhals in the West Greenland area exist have been tested by different tools (metal and organochlorine concentrations, stable isotopes and DNA).

Metal analysis

In total, Cd, Hg, Se and Zn concentrations in muscle, liver and kidney tissues of app. 150 narwhals were analysed. Cd, Hg, Se concentrations were increasing in the first three to four years of the animals life, where after no dependence on age was observed. Females had significantly higher concentrations than males in case of Cd in all tissues and Hg and Se in liver. No consistent difference in metal levels between narwhals from Avanersuaq and Uummannaq was found. The between year variation at one location seem to be larger than the variation between the two location. The metal levels found were within the range of previous published results on narwhals from Arctic Canada and Avanersuaq, West Greenland.

Organochlorines

In total, organochlorine (POP) concentrations in blubber from 101 narwhals were analysed. POP concentrations were dependent on age and differences were found between males and females. Females showed decreasing POP concentration in the first 8 to 10 years, while for males small changes were observed in the first few years and POP concentrations were rather independent of age. No or few statistical differences in mean POP concentrations were observed, however, the narwhals from Balgoni Island 1993 and Kitsissuarsuit 1990 showed the consistently highest levels and whales from Avanersuaq 1993 the lowest level. Also the pattern of PCB congeners showed some differences between samples. PCBs seem to be at a little lower or at the same level in the West Greenland samples as in the Canadian samples, whereas HCHs and DDTs seem to be higher. Total toxaphene was one of the dominant POPs as previously observed in small Arctic toothed whales, but showed no significant geographical differences.

Stable isotopes

Stable isotope ratios in muscle of 150 narwhals were analysed. The ratios showed a decreasing trend in the first year when they live of mother's milk, hereafter the ratios were relative stable with age. $\delta^{15}N$ was found to be significantly higher in samples from Uummannaq 1993 compared to the samples from Avanersuaq 1984 and 1985 indicating some difference in tropic levels of the narwhals. $\delta^{13}C$ was also found to be significant higher in samples from Uummannaq 1993 than from Avanersuaq 1985 but not from Avanersuaq 1984. Only a few significant correlations were found between stable isotopes ratios and metal and POP concentrations.

Genetic data

Genetic haplotypes in the skin of 426 narwhals were analysed from the same regions covered by the other analysis. Additional 99 samples were analysed from Disko Bay, Balgoni Island, Eastern Canada and Eastern Greenland. In general, the genetic variation was low relative to that found for other cetaceans. However, the genetic data gave the strongest indications of the occurrence of different populations of narwhals in the West Greenland area expressed by the frequencies of haplotypes.

Conclusion

The population structure with two West Greenland populations suggested by the genetic study could not be supported by the metal and POP concentrations and staple isotopes.

Eqikkaaneq

Akuutissanik arrortikkuminaatsunik qanoq akoqartiginersut, akuutissanik organoklorininik (klorimik akulinnik) qanoq mingutsinneqarsimatiginersut, sunik nerisaqartarnersut aammalu uumasut sananeqaataasa ilisarnaataat (DNA) paasiniarlugit kitaaniittut qilalukkat qernertat ujaluinik, tinguinik, tartuinik, orsuanik mattaanillu misissuineq ingerlanneqarpoq.

Aatsitassat arrortikkuminaatsut DNA-mullu tunngasut paasisat nutaat ilisimasanut pigeriikkanut tapertaalluarput. Mingutsitsisartulli organoklorinit aammalu sunik nerisaqarnerannik misissuinermi uuttorneqartartut stabile isotopet pillugit paasisat nutaajupput.

Taakkulu assigiinngitsut sisamat atorlugit paasiniarneqarpoq kitaaniittut qilakkut qernertat uumasoqatigiiaat arlariiunersut.

Arrortikkuminaatsut misissornerat

Qilalukkat qernertat 150-it ujalui, tingui tartuilu ukuninnga qanoq akoqartiginersut misissorneqarpoq; Cd, Hg, Se aamma Zn. Ukiut uumaffiusut siulliit 3-4-mat ingerlaneranni Cd-mik, Hg-mik Se-millu uumasut akoqarnerat annertusiartortoq tamatuma kingorna utoqqaassuseq apeqqutaajunnaartarpoq. Cd (ujaluni tamani) aamma Hg Se-lu (tingummi) akuusoq tiggannut sanilliullugu arnaqquassaani annerusarpoq. Qilalukkat qernertat Avanersuup Uummannallu eqqaanniittut aatsitassanik arrortikkuminaatsunik akui nikingangaanngillat. Sumiiffiit taakku akornanni nikingassutaasut annikinnerusutut ippoq illuatungaani sumiiffimmi immini ukiumiit ukiumut allanngoriarneq annerusutut isikkoqartoq. Canadap avannaarpiarsuani Avanersuarmilu qilalukkat qernertat siusinnerusukkut misissuiffigineqarneranni aatsitassanik arrortikkuminaatsunik nassaat annertussusaat maanna misissuinermi nassaanit allaanerunngilaq.

Organokloriner

Qilalukkat qernertat 101-it orsuinik misissuinermi paasiniarneqarpoq akuutissat mingutsitsisartut organoklorinit (POP) qanoq annertutiginersut. POP-it qanoq annertutiginerannut uumasut qanoq utoqqaatiginerat apeqqutaavoq – aammalu angutiviaanersut arnaviaanersulluunniit apeqqutaasarluni. Ukiut 8-10-t siulliit ingerlaneranni akuutissat POP-it arnavissani naammattoorneqartut annikil-

liartortarput illuatungaani angutivissani ukiut siulliit annikitsumik allanngortarsinnarlutik kingorna utoqqaassusaat apeqqutaajunnaartarluni.

Akuutissat mingutsitsisartut POP-ip nassaarineqartut agguaqatigiissinneranni nikingassutaasoq annikitsuinnaavoq. Balgoni Islandimili (1993) kiisalu Kitsissuarsunni (1990) misissuinermi qilalukkat qernertat POP-inik annerpaamik akoqartut Avanersuarmi (1993) akoqannginnerpaapput. Akuutissat mingutsitsisartut ilaat PCB-congen misissuinerit akornanni annertussutsimigut allanngorarpoq. Kitaani nassaat PCB-it, canadamiut misissuineranni nassaarineqartutuulli, annikinnerusut illuatungaani HCH-mik DDT-millu nassaat anneruppu t. Issittumiittut arferit kingutillit minnerit misissuiffigisarneranni total toxafen naammattuugassaanerusarpoq sumiinneralli apeqqutaannginnerulluni.

Stabile isotoper

Qilalukkat qernertat sunik nerisaqarnerannik misissuinermi stabile isotoper uuttorneqartarput, misissuiffigineqarpullu qilalukkat qernertat 150-it ujalui. Arnamik immuanik inuussuteqarnerisa nalaanni ukiut siulliit stabile isotopet annikilliartortarput kingorna allanngorarpiaratik utoqqaassusaallu apeqqutaajunnaartarluni. $\delta^{15}N$ Uummannami (1993) misissuinermi nassaarineqartoq 1984-mi 1985-milu Avanersuarmi uuttorneqartumut sanilliullugu annerungaatsiarpoq - tamatumalu pasinarsisippaa uumasut imaaniittut imminnut nerisareqatigiinneranni pissutsit assigiinngissuteqarsinnaasut. $\delta^{13}C$ eqqarsaatigalugu Uummannami (1993) nassaarineqartoq Avanersuarmi (1985) nassaarineqartumik qaffasinnerungaatsiarpoq, kisiannili tamaani 1984-mi nassaarineqartunik allaaneruallaarani. Stabile isotopet, aatsitassat arrortikkuminaatsut akuutissallu POP-it sunneeqatigiittarnerat annikitsuinnaavoq.

Timitaasa ilisarnaataat

Qilalukkat qernertat 426-it mattaanik misissuinerni paasiniarneqarpoq timitaasa ilisarnaataat (DNA), misissuinernik ingerlatsiviusimasut sumiiffiit allanngortinneqaratik. Misissugassat allat 99-it Qeqertarsuup Tunuanit, Balgonip Qeqertaanit, Canadap kangianit Tunumiillu pissarsiarineqarput misissorneqarlutik. Arfernut allanut sanillersuutissagaanni qilalukkat qernertat timitaasa ilisarnaataat allanngorarpianngilaq. Taamaakkaluaq paasisat ilimanarsisippaat kitaaniittut qilalukkat qernertat uumasoqatigiiaat arlaqartut.

Inerniliineq

Qilalukkat qernertat timitaasa sananeqaataannik misissuinerit ilimanarsisikkaluaraat miluumasut taakku kitaaniittut uumasoqatigiit arlariiusut taamaattoq ilimatsaassineq taanna ilumut taamaannersoq aatsitassanik arrortikkuminaatsunik, akuutissanik POP-inik aammalu nerisaasa suunerannik misissuinermi uuttuutinik stabile isotopenik paasisat uppernarsaatitut atorneqarsinnaanngillat.

Resumé

Prøver af muskel, lever, nyre, spæk og hud fra vestgrønlandske narhvaler er blevet analyseret for tungmetaller, organokloriner, stabile isotoper og DNA.

De opnåede resultater for tungmetaller og DNA supplerer eksisterende data, mens ingen tidligere data eksisterer for organokloriner og stabile isotoper i vestgrønlandske narhvaler.

En hypotese omkring eksistensen af forskellige populationer af narhval i det vestgrønlandske område er blevet testet ved anvendelse af forskellige biomarkør-værktøjer (metal og organokloriner, stabile isotoper og DNA).

Metal-analyser

Der er blevet analyseret for Cd, Hg, Se og Zn i muskel, lever og nyre fra ca. 150 narhvaler. Cd, Hg og Se koncentrationerne var stigende i de første 3-4 år leveår, hvorefter ingen aldersafhængighed var synlig. Hunner havde signifikant højere koncentrationer end hannerne når det gjaldt Cd (alle væv) og Hg og Se (lever). Der blev ikke fundet konsistente forskelle i metal-niveauerne mellem narhvaler fra Avanersuaq og Uummannaq. For en lokalitet syntes år til år variationen at være større end variationen mellem de to lokaliteter. De fundne metal-værdier var på niveau med tidligere publicerede resultater på narhvaler fra Arktisk Canada og Avanersuaq, Vestgrønland.

Organokloriner

Der er blevet analyseret for koncentrationer af organokloriner (POP) i spæk fra i alt 101 narhvaler. POP koncentrationer var aldersafhængige, og afhængigheden var forskellig for hanner og hunner. Hos hunnerne var POP koncentrationer faldende i de første 8-10 år, mens hannerne viste beskedne ændringer i de første år og dernæst umiddelbart uafhængig af alder. Ingen eller få statistiske forskelle i middel POP koncentrationer blev fundet, dog viste narhvaler fra Balgoni Island 1993 og Kitsissuarsuit 1990 konsistent de højeste niveauer og hvaler fra Avanersuaq 1993 det laveste niveau. Ligeledes viste PCB-congen mønstret nogle forskelle mellem prøverne. PCB-koncentrationer i Vestgrønland var lidt lavere eller på samme niveau som i canadiske prøver, hvorimod HCH og DDT-koncentrationer var højere. Total toxafen var en af de dominante POP'er som tidligere observeret i små arktiske tandhvaler, men viste ingen signifikante geografiske forskelle.

Stabile isotoper

Stabil isotop ratioer i muskel blev analyseret i 150 narhvaler. Ratioerne viste en aftagende trend i de første år hvor de ernærer sig af modermælk, hvorefter ratioerne var relative stabile og aldersuafhængige. $\delta^{15}N$ var signifikant højere i prøver fra Uummannaq 1993 sammenlignet med Avanersuaq 1984 og 1985, hvilket indikerer nogle forskelle i trofisk niveau mellem disse narhvaler. $\delta^{13}C$ blev ligeledes fundet, at være signifikant højere i prøver fra Uummannaq 1993 sammenlignet med Avanersuaq 1985, men ikke for Avanersuaq 1984. Kun få signifikante korrelationer blev fundet mellem stabil isotop ratioer og metal og POP koncentrationer.

Genetiske data

Genetiske haplotyper i huden fra 426 narhvaler blev analyseret fra samme regioner som dækket af de andre analyser. Yderligere 99 prøver fra Disko bugt, Balgoni øer, Østcanada og Østgrønland blev analyseret. Den geniske variation var generelt relativ lav sammenlignet med andre hvaler. Trods dette, gav de genetiske data den stærkeste indikation for eksistensen af forskellige narhvalpopulationer i det vestgrønlandske område udtryk som frekvenserne af haplotyper.

Konklusion

Populationsstrukturen med de to Vestgrønlandske populationer, som det genetiske studie antydede, kunne ikke understøttes af metal og POP koncentrationer og stabile isotoper.

1 Introduction

Objective

Contaminants are being studied in a large number of species throughout the World including the Arctic. Within the contaminant studies a number of questions are being dealt with including e.g. pathways, bioaccumulation, age accumulation, geographical and temporal trends. Observed similarities and differences are often explained from information of general feeding habits and trophic position. In some cases a linkage from the individual animal to its contaminant burden has been tried to be inferred from the stomach content analysis (e.g. Muir et al. 1995). However, stomach content mainly reflects recent food intake and may not necessarily reveal the long-term feeding habit (Atwell *et al*. 1998).

The measurement of stable isotopes of nitrogen $\delta^{15}N$ (^{15}N /^{14}N) has proven to be useful way of explaining differences in trophic levels as there is a relative enrichment of ^{15}N in the order of 3-5 ‰ at each trophic transfer (Hobson and Welch 1992, 1995). Carbon Isotope ratios ^{13}C /^{12}C ($\delta^{13}C$) usually show much less trophic enrichment and can be used to infer food source (Hobson and Welch 1992, 1995). A number of studies have documented the general accumulation on a large number of different species (e.g. Hobson *et al.* 1997; Atwell *et al.* 1998), whereas other studies deal with species-specific differences (e.g. Hobson and Welch 1995; Muir *et al.* 1995; Smith *et al.* 1996).

The West Greenland narwhals were chosen for this study, as we hypothesise, that two sub-populations of narwhals are found in the West Greenland area based on previous results on telemetry and DNA analysis (Dietz and Heide-Jørgensen 1995; Palsbøll *et al*. 1997). The hypothesis is that one population summers in the Melville Bay area and winters outside Disko Island and another population stays in the northern Baffin Bay (east Canada and Avanersuaq District) and pass through Uummannaq in central Davis strait on their way to their wintering grounds. The heavy metal, organochlorine and staple isotope levels in tissues of these two populations were analysed in order to test whether they support the hypothesis. Furthermore, DNA in skin samples from narwhals was determined to supplement the existing DNA analysis and conclusions of Palsbøll (*et al*. 1997) .

2 Materials and Methods

2.1 Sampling techniques and storage

Table 2.1.1 gives an overview of narwhal samples, and figure 2.1.1 shows a map of sampling locations.

In 1984 and 1985 the majority of samples from Avanersuaq were collected by the hunters due to the scattered distribution of the hunt,

making it difficult for biologists to obtain samples. In addition to the samples of muscle, liver, kidney, blubber, skin, jaw and the embedded tusk the hunters provided information on date, location, sex, zoological body length, and a field estimate of the animal's age.

Samples from the other areas were collected by biologists. In most cases the lower jaw and the imbedded tusks were sampled for later age determination. Muscle, liver, kidney were collected for metal analysis, whereas blubber and skin samples were sampled for POP and DNA analysis. Stable isotope was analysed from the muscle samples.

The samples were stored in polyethylene plastic bags and maintained at outdoor temperatures (approximately –30 to +5 °C) until stored in a freezer (-20°C). The tissues remained at -20 °C during shipment from Greenland and storage at Department of Arctic Environment, National Environmental Research Institute (DAE) Roskilde, Denmark.

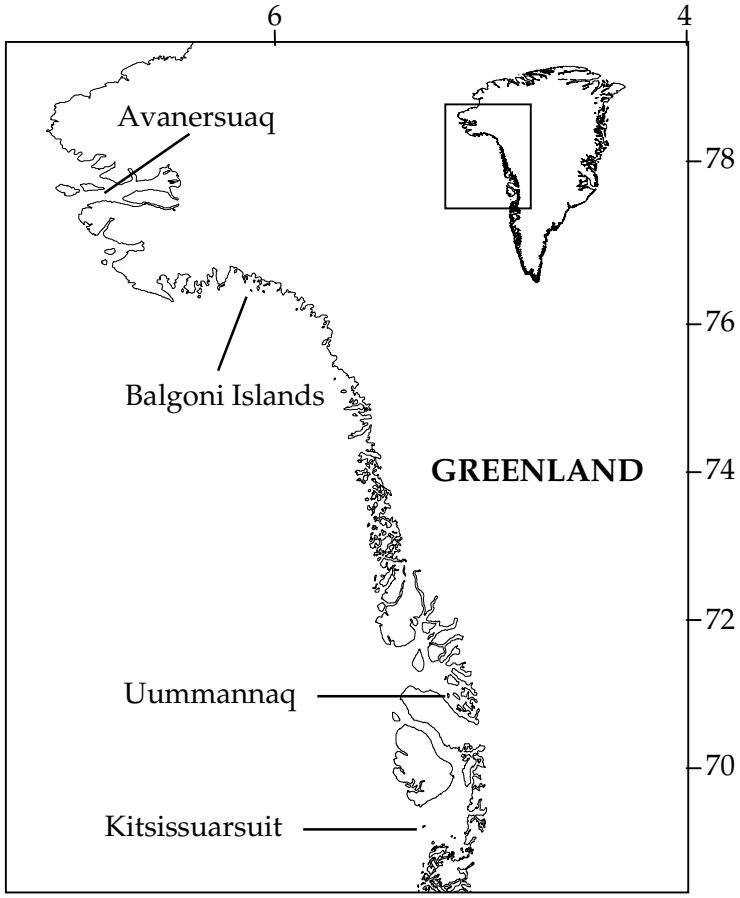

Figure 2.1.1. Map of sampling localities.

Table 2.1.1. Overview of number of analysed narwhal samples.

Location	Year	Element	Muscle	Liver	Kidney	Blubber	Skin
Avanersuaq	1984	Metals	50-55	52-54	54-55		
		POP's				4	
		Stable isotopes	50				
		DNA					55
Avanersuaq	1985	Metals	24-33	29-32	31-34		
		POP's				26	
		Stable isotopes	33				
		DNA					35
Avanersuaq	1993	Metals	4	1	1		
		POP's				4	
		Stable isotopes	4				
		DNA					135*
Uummannaq	1983	Metals	4	4	4		
		POP's					
		Stable isotopes					
		DNA					74**
Balgoni Island	1993	Metals	6	6	6		
		POP's				6	
		Stable isotopes	6				
		DNA					20*
Kitsissuarsuit	1990	Metals	3	2	2		
		POP's				2	
		Stable isotopes	2				
		DNA					3
Kitsissuarsuit	1994	Metals	2	2			
		POP's				2	
		Stable isotopes	2				
		DNA					51

* 1993 and 1994
** and other years se also Table 3.5.1 for additional samples

2.2 Age determination

Age estimates from the embedded tusk

The right embedded tusk was used to examine the presence of growth layers in the internal dentine and in the cementum formed at the posterior apex of the embedded tusk. After removal from the freezer the embedded tusks were cleaned with tap water and a sponge to remove blood, lipid and connective tissue. In addition the animals as a minimum could be categorised into groups of: newborn and yearlings, based on the macroscopic appearance of the imbedded tusk following the method described by Hansen *et al*. (1990). The posterior 2-cm of the tooth tip was cut off using a diamond saw (Buehler Isomet). A diamond saw likewise bisected the rest of the tusk in the mid-sagittal plane.

Examination of the dentine

The surface of the bisected tusk was polished on 320-grade sandpaper to remove the saw marks. The primary layering of the dentine

was marked up and counted by the naked eye or by use of a binocular microscope. Further storage of the tusk was done in hermetically sealed plastic bags with a bit of ethanol to prevent cracking from drying out as well as bacteria and algae formation.

Examination of the cementum

The tusk tip was decalcified in a 5% HNO_3 solution for 2-3 days as described by Grue and Jensen (1979). The decalcified tusk tip was imbedded in Tissue Tek.® at –20°/-22°C on the freezing platform of a Reichert Jung freeze microtome (1206) cooled by a Reichert Jung Frigomobil cooling unit (OM1206). Central longitudinal thin sections of 12-14µ were made using a type D cutting blade. Five of the best sections from each tooth were placed in water with 5% (by weight) gelatine powder. The sections were placed on glass slides and dried at room temperature for at least 24 hours. The sections were stained with toulidine blue using the methods described by Dietz *et al.* (1991). Excess colour was removed by three fresh water baths and a dehydration sequence from 70 over 96% to 2x99% C_2H_5OH ending in xylene or toluene. Permanent mounting was performed using Entellan and cover slips. The growth layer groups of the cementum was counted under a binocular Leitz microscope equipped with phase contrast and blue filters. It is generally recognised that one GLG in mammls equals one year (Perrin and Myrick 1980; Grue and Jensen 1979) and although this has never been verified for narwhal, we chose to let one Growth Layer Group represent one year of age.

2.3 Metal analysis

The metal analyses were performed at the Department of Arctic Environment laboratory (DAE). After removal from the freezer the tissue samples were lightly thawed and the outer exposed tissue layer was cut away to minimise possible contamination and changes in water content due to handling and storage. Stainless steel scalpels, polyethylene gloves, and cutting boards were used. Approximately 0.5 g of tissue was transferred to the tarred Teflon liner of a Berghof stainless steel bomb. After the addition of 3 ml of 65% HNO_3 (Merck Suprapur®), the bombs were closed and incubated for twelve hours at 120° -150°C. Following a cooling period, the digests were transferred quantitatively to 50 ml screw-cap polyethylene bottles and adjusted to c. 25 g weight using metal-free, deionized water (Millipore®). Approximately 8% HNO_3 was used for all further dilutions.

All zinc analyses were carried out by flame AAS (Perkin-Elmer 3030). All cadmium samples were screened using the same technique, however, the graphite furnace technique (Perkin-Elmer 3030 with Zeeman background correction) was used for the final analysis of low concentration (2.5 µg/g wet weight). This latter technique was always used for selenium analyses. Mercury analyses were performed using hydride generation and the amalgam technique, as described by e.g. Nielsen and Dietz (1990).

The detection limits were 0.015, 0.005 and 0.20 µg/g wet weight for laboratory analyses of cadmium, mercury and selenium, respectively. All concentrations are reported as µg/g wet weight (ww). For recalculation into µg/g dry weight, the factors 3.66 (muscle), 3.17 (liver)

and 3.67 (kidney) were computed from the means of weight percentages routinely recorded in the DAE laboratory.

The analytical quality was checked by repeating analyses, and by the frequent use of various reference standards; especially Tort-1 (lobster hepatopancreas) supplied by the National Research Council of Canada (Marine Analytical Chemistry Standards Program) and the dried tuna internal standard of National Food Agency of Denmark. The DAE laboratory participates in the international intercalibration exercises conducted by the International Council for the Exploration of the Sea (ICES), EEC (QUASIMEME), National Research Council, Canada and by the Dept. of Fisheries and Oceans, Winnipeg, Canada (see Asmund and Cleemann 2000).

2.4 PCBs and selected organochlorine pesticides

PCBs (CB-28, CB-31, CB-52, CB-101, CB-105, CB-118, CB-138, CB-153, CB-156, and CB-180) and selected organochlorine pesticides (HCB (hexachlorobenzene), α-HCH, β-HCH, p,p'-DDE, p,p'-DDD and p,p'-DDT, and trans-nonachlor) have been analysed in 50 narwhals by The National Environmental Research Institute, Department of Environmental Chemistry (NERI) and in 44 narwhals by RIVO-DLO, Ijmuiden, The Netherlands (RIVO). Total toxaphene (chlorobornanes) and the individual chlorobornanes: CHB-26, CHB-32, CHB-50 and CHB-62 were all analysed by RIVO.

2.4.1 NERI analysis methods

PCBs and organochlorine pesticides were analysed using the method described in AMAP- Greenland (1994-1996), section C, Appendix 3 (Aarkrog et al. 1997).

A sample of blubber (0,5 g) was homogenised and grinded in a mortar with a sufficient amount of sodiumsulphate to dry the sample. The dried sample was transferred to a Soxhlet insert and Soxhlet extracted for 6 hrs with a mixture of hexane+acetone, 4:1. 10 % of the extract was used for lipid determination. The rest was concentrated on a rotary evaporator and cleaned up on multilayer column consisting of silica impregnated with sulfuric acid, silica and alumina with 10 % of water and eluted with hexane. The hexane extract was concentrated and analysed with and without internal standards on a gas chromatograph equipped with a dual column system and ECD-detectors. The concentrations were determined using a six level calibration curve.

The 50 samples were originally divided in 4 sample series. Samples from the two districts were randomised in such a way that samples from both districts was analysed in every batch in an equal amount. In every batch an internal reference material consisting of sand eel oil in duplicate, one real sample in duplicate and a blank were analysed. All blank values were below the limit of detection.

Before extraction a mixture of three standards (CB-3, CB-40 and CB-198) were added to each sample in order to monitor the extraction

efficiency. A good correlation between recovery of the spiked standards and the compounds analysed has been found (Asmund and Cleemann 2000). Samples with recoveries below 80 % for CB-40 and CB-198 were reanalysed. The recovery of CB-40 and CB-198 is given with the results of each sample in appendix 6. CB-3 is a rather volatile compound and the recovery is used as an indicative value only.

The laboratory participated in a QUASIMEME intercomparison prior to the beginning of the analysis and during the analysis, both times with excellent results.

2.4.2 RIVO analysis methods

The dry weight percentage and the total lipid content were also determined in the narwhal blubber samples.

The extraction and clean-up procedure for the blubber samples was comparable to that described by de Wit et al. (1997). The blubber was homogenized with a knife and a portion of the sample was gently melted. The obtained oil was weighed and dissolved in n-pentane. An aliquot containing not more than 250 mg fat was transferred to a 15 g alumina column (9% H_2O). This column was eluted with 170 ml pentane to collect the PCB and pesticide fraction, while the fat remained at the column. A portion of 500 mg fat was transferred to the top of a 25 g alumina (10% H_2O) column and eluted with 250 ml pentane to collect the toxaphene fraction, while the fat remained at the column. Both pentane volumes were concentrated on a rotary evaporator after addition of 2 ml iso-octane to each of them as a keeper. The remaining iso-octane solutions were aliquots used for the fractionation of toxaphene and the CHBs. This aliquot was transferred to the top of a silica column (2.5 g SiO_2.2% H_20 and eluted with 13 ml iso-octane. This fraction contained ca. 35% of CHB 26 and ca. 5% of total-toxaphene. A second fraction was eluted with 12 ml 20% diethylether in iso-octane (v/v). This fraction contained the rest of CHB 26 and total-toxaphene. Another silica gel elution was carried out prior to the determination of PCBs and pesticides. The remaining 2 ml were transferred to the top of a 1.6 g SiO_2.2% H_20 column. Elution took place with 11 ml iso-octane (PCBs, p,p'-DDE and HCB) and 10 ml 15% diethylether in isooctane (remaining pesticides).

Total-toxaphene and the CHBs were determined by GC/MS, using negative chemical ionisation (NCI). The other analytes were determined by GC/ECD (electron capture detection). Multi-level calibration was carried out with 6 different levels for the total toxaphene, and 8 levels for the CHBs. Multi-level calibration was carried out with five different levels for GC-ECD analysis.

Because chlordane cannot be separated from toxaphene by prefractionation, the total toxaphene results, which were obtained by comparing the area under all peaks in the technical toxaphene mixture and that under all peaks in the total ion chromatogram of the samples, were corrected for the area of the chlordane peaks.

Internal reference materials - cod liver for PCBs and pesticides and hake liver for toxaphene - were included in each series of ca. 15 sam-

ples. Additionally, two certified reference materials (CRMs) - mackerel oil (BCR 350) - was analysed for PCBs and a cod liver oil (BCR 598) was analysed for pesticides. A blank was included in each sample series and recoveries were measured for all analytes in each series by means of standard solutions, which were subjected to all steps of the analytical procedure. Within the QUASIMEME programme, twice per year two unknown samples are analysed for PCBs and organochlorine pesticides, and once per year for toxaphene congeners.

RIVO-DLO is accredited for PCB, organochlorine pesticide and total-toxaphene analysis (Accreditation no. L097, Sterlab, The Netherlands).

2.5 Stable isotope analysis

Stable-nitrogen and stable-carbon isotope ratios were measured in samples of skeletal muscle from a total of 151 individuals according to Hobson et al. (1997). In short the muscle tissue was freeze-dried then powered in an analytical mill. Lipids were removed using a modified extraction technique based on the Bligh and Dyer method (1957) and the samples dried at 60°C for >24 hours. Samples were combusted according to the Dumas method (see Knowles and Blackburn 1993) and then allowed cooling slowly in the furnace for 18 hours. CO_2 and N_2 gas were separated cryogenically and analysed using a VG OPTIMA isotope ratio mass spectrometer. The standards for stable isotope ratio analysis were atmospheric air (AIR) for nitrogen and Pee Dee belemnite (PDB) for carbon.

2.6 DNA

2.6.1 Extraction

DNA extractionStandard phenol/chloroform extraction. Samples were delivered prepared in 400 µL extraction buffer (0.1 M NaCl, 0.05 M EDTA, 0.1 M Tris-HCl, pH 8.0 and 1% SDS). To this solution 40 µL Proteinase K (10 mg/ml) was added and digestion allowed to take place over night at 65°C. The following day the aquatic phase was extracted one time with 200 µL equilibrated phenol (pH 7.6) and 200 µL isoamylalcohol:chloroform (1:24), twice with 400 µL isoamylalcohol:chloroform (1:24), and finally precipitated by addition of 1 ml 96% ethanol and centrifugation at 13,000 x *g*. The precipitated DNA was washed once with 500 ul 70% ethanol and dried at 65°C after which it was re-suspended in 1 x TE (0.1 M Tris-HCl, pH 8.0, 0.05M EDTA.

2.6.2 Restriction fragment length polymorphism (RFLP) analysis

MtDNA primers

Restriction endonuclease digestion with the restriction endonucleases (RE) *Mnl*I and *Bgl*II was carried out as described in Palsbøll *et al.* (1997). The 3' end of the mitochondrial control region was amplified by PCR™ using the primers MT4 (5'-CCTCCCTAAGACTCAAGGA

AG-3', (Arnason *et al.* 1993)) and Mn312 (5'-CGTGATCTAATGGAGC GGCCA-'3, (Larsen *et al.* 1996).

Polymerase chain reaction (PCR) amplification

The PCR™ amplifications were carried out in 20 µl volumes with 20 mM Tris-HCl (pH 8.4), 50 mM KCL, 2 mM $MgCl_2$, 0.05 units of *Taq* DNA Polymerase (Life Technologies Inc.) and 1 mM of each primer. In total 29 cycles amplification cycles were performed with one min. at 94 °C, 1 min. at 54 °C and 4 min. at 72 °C on a Stratagene RoboCycler™.

Redigestion

After amplification 5 µL aliquots were each digested with either the restriction endonuclease *Mnl*I and *Bgl*II under the conditions recommended by the manufacturer (New England Biochemical, Inc.).

Detection

After completed digestion the restriction fragments were separated by horizontal electrophoresis in 2% agarose. The restriction fragment pattern were visualised by ethidium bromide staining and documented with a Pharmacia ImageMaster VDS™ system.

2.6.3 Nucleotide sequence determination

Direct nucleotide sequencing

The sequence of the first 287 nucleotides in the mitochondrial control region was determined by standard protocols using fluorescent technology. The initial symmetrical PCR™ amplification was performed as described for the RFLP analysis. The subsequent fluorescent-labelled single-stranded products were generated by PCR™ using the ABI Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (by PE Applied Biosystems).

Detection of sequencing products

The labelled fragments were separated by electrophoresis through a 5% denaturing Long Ranger™ (FMC, Inc.) sequencing matrix on an ABI Prism™ 377 automated sequencer. The reverse strand was sequenced in samples that yielded an ambiguous or new sequence.

2.6.4 Population genetic analysis

Nucleotide diversity

The degree of genetic variation was estimated as the nucleotide diversity (Nei 1987), which is an estimate of the average number of polymorphic nucleotides per nucleotide among the sampled sequences.

Genetic divergence

The degree of genetic divergence between sample partitionings was estimated as H_{ST} (Hudson *et al.* 1992). Monte Carlo simulations estimated the probability of observing the estimated level of genetic divergence if the two sets of sequences were collected from the same population. In each simulation, the sampled sequences were randomly assigned to one of the two population samples in the original proportions and the level of genetic divergence estimated (as H_{ST}).

Significance tests

A total of one thousand simulations were performed per test and the probability of the observed value of H_{ST} was estimated as the proportion of simulations that yielded a similar of more extreme (=higher) estimate of H_{ST} (Hudson et al. 1992).

Estimation of genealogical relationship

A genealogy of the haplotypes (each unique sequence) and the reproducibility of each node was estimated using the PHYLIP 3.5c computer package (Felsenstein 1993). The homologous sequence from the beluga, *Delphinapterus leucas*, was used to define the position of the root of the genealogy. One thousand bootstrap samples were generated from the original data set with the program SEQBOOT (Felsenstein 1993). The genealogy of each bootstrap sample was estimated by use of DNADIST (Felsenstein 1993). Kimura's two-parameter distance (Kimura 1980) and NEIGHBOR (Felsenstein 1993) (Neigborjoining method, (Hasegawa & Fijuwara 1993) with jumble option on). A majority consensus rule genealogy was obtained from the 1,000 genealogies was obtained by use of CONSENSE (Felsenstein 1993).

Estimation of population genealogy

A genealogy of the population samples defined by the homogeneity tests (H_{ST} levels of genetic divergence above) was estimated using the programs GENDIST, NEIGHBOR and CONSENSE (Felsenstein 1993) from 1,000 bootstrap samples. Each bootstrap sample was generated by resampling individual sequences from the original data set *with* replacement and in the original proportions and the population frequency of each allele estimated (program by P.J. Pallsbøl).

2.7 Data treatment

Growth curve

The parameters asymptotic length and the constants b and k of Gompertz growth model have been estimated by non-linear regression.

Correlations

Pearson's product-moment correlation has been applied in correlations analysis.

Data presentation

Arithmetic mean values is chosen to be presented instead of geometric mean values since arithmetic values are unbiased and more meaningful in studies on levels of contaminants in the environment as recently outlined by Parkhurst (1998). However, before testing of differences in mean concentrations between locations, data were log-transformed in order to reduce skewness and meet the assumptions of parametric statistical tests.

Metals

A two-way analyses of variance (ANOVA) including the effects of location (combination of location and sampling year) and sex together with their interaction effect has been used. If the interaction effect showed up to be non significant at the 5% level *post hoc* Tukey's studentized range test was applied to test for differences in mean values between locations and sex. However, both the ANOVA and the Tukey's studentized range test have problems when having very unequal sample size (SAS 1999). In these data the sample size of females from Avanersuaq in 1985 and 1993 and of males from Uummannaq 1983 are very small compared to the other sample size. Although, these tests are recommended for use in cases with unequal sample size, the tests lead to counter-intuitive results in a few cases. Therefore, the results from these tests have to be interpreted carefully.

POPs

POP concentrations were expressed on a lipid basis because the compounds are highly lipophilic.

An analysis of covariance (ANCOVA) was used including locations (combination of location and year) and sex as factors and age as a covariate. The first order interactions effects between all factors and covariate were also included. The statistical ANCOVA model was successively reduced for factors not significant at the 5% level according to Type III sum of squares test starting with the interactions factors. A main factor (location, sex and age) was not removed from the model if it was included in a significant interaction factor. It showed up that age was a significant covariate and sex a significant factor for all POP groups (see later). Therefore, the final reduced statistical model was used to test for differences in age adjusted mean POP concentrations between localities by testing of the least square mean (LSMEAN) values.

The estimated age adjusted mean and variance (var) of POP concentrations were back transformed to an arithmetic scale by the following formulas:

$$meanPOP = \exp(\alpha + \frac{\beta^2}{2})$$

$$\text{var } POP = \exp(2\alpha + \beta^2) * (\exp(\beta^2 - 1)$$

Where α and β are the mean and variance on a logarithmic scale, respectively.

The pattern of PCB congeners has been analysed with a principal component analysis (PCA). Since the analysis is concerned with describing the pattern rather than the general level of PCB congeners the congener concentrations were normalised to have the sum 1 prior to the analysis:

$$x_{ij} = \frac{x'_{ij}}{\sum_{k=1}^{m} x'_{ik}}, i = 1\ldots\ldots\ldots, n$$

where: x'_{ik} denotes the level of the jth PCB congener
m denotes the number of PCB congeners
n the numbers of narwhals

The PCA was performed on the correlation matrix and the principal components were VARIMAX rotated in order to facilitate the interpretation. The differences in mean principal scores were analysed by a one-way analysis of variance (ANOVA) followed by the *post hoc* Tukey's studentized range test.

Stable isotopes

The ratio $\delta^{15}N$ and $\delta^{13}C$ were analysed following the same procedure as described for the metals except that no log-transformation was done ($\delta^{15}N$ and $\delta^{13}C$ followed approximately a normal distribution).

Software

*T*he statistical analyses were performed with the SAS statistical software package (SAS, Version 8.2).

3 Results and discussion

3.1 Age determination and growth curves

Missing age

The age of 18 individuals (14 females and 4 males) was missing because of lack of collected teeth. In order to estimate an age of these individuals the growth curve for each sex was estimated.

Growth curve

A growth curve was estimated for each sex using a Gompertz growth model:

$$SL = L_{\bullet} * \exp(-b * \exp(-k * Age))$$

where: SL = standard length

$L_{\bullet}$ = the asymptotic standard length

b, k = constants

Age = age of the narwhals

$L_{\bullet}$, b and k were estimated by non-linear regression and the final growth curves were :

Males: $SL = 475 * \exp(-0.97 * \exp(-0.25 * age))$, $R^2 = 0.91$

Females: $SL = 406 * \exp(-0.83 * \exp(-0.42 * age))$, $R^2 = 0.93$

The male narwhal has a larger asymptotic length than the female narwhal. In addition, the males reach its asymptotic length at a higher age than the females (Figure. 3.1.1).

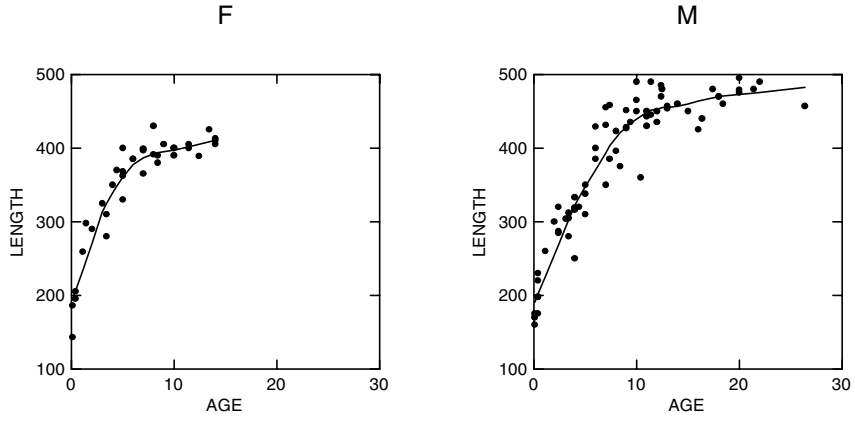

Figure 3.1.1. Growth curve of female (F) and male (M) narwhals. The lines are LOWESS smoother.

Previous studies

The estimated asymptotic length and the constants (b and k) were very similar to those found in a study in 1993 and 1994 of 108 narwhals from Ingefield Bredning, Uummannaq and Disko Bay (Neve 1995).

Age distribution

The age distribution of the collected narwhals is shown in Table 1. No female older than 14 years was found, while males up to 26 years old was found. In general, the males from Uummannaq are older than the females and also older than the males from Avanersuaq.

3.2 Metal analysis

Table 3.2.1 shows the descriptive statistics of metal concentrations in muscle, liver, kidney tissues of narwhals for comparably purposes. It is well established that metal concentrations may be dependent on age and in some case also sex. It is therefore necessary to take these factors into account when comparing metals levels between locations and/or years.

3.2.1 Influence of age on metal concentrations

The relationship between metal concentration and age of narwhal is shown in Figure 3.2.1 for each metal and tissue combination. The aim of Figure 3.2.1 is only to present a general pattern of the relationships illustrated by use of the LOWESS smoother. Therefore, no attempt was made in this Figure to elucidate the dependency of location or sex of the whales.

Cd, Hg and Se

Cd, Hg and Se concentrations in muscle, liver and kidney tissue showed a similar pattern with an increasing of concentrations in the first 3 to 4 years of the narwhals life, and thereafter a relatively constant level of concentrations (in case of Cd a slightly decrease) in the rest of their life. However, Se concentrations in muscle did not quite follow that pattern.

The increasing of Cd and Hg supports previous findings by Wagemann et al. (1983, 1990, 1996) of whales from Canadian waters and Hansen et al. (1990) of minke whales (*Balaenoptera acutorostrata)*, belugas *(Delphinapterus leucas)* and narwhals from Greenland.

Zn

Zn concentrations in muscle, liver and kidney tissue showed a different pattern from that of the other metals. Zn concentrations have a relatively constant level of concentrations during the whole life and seemed therefore not to be dependent on the age of the narwhals.

Hansen (1990) and Wagemann et al. (1983) likewise found a lack of age accumulation of Zn in narwhals. As Zn is an essential metal, and the body is capable of regulating the physiologically required levels.

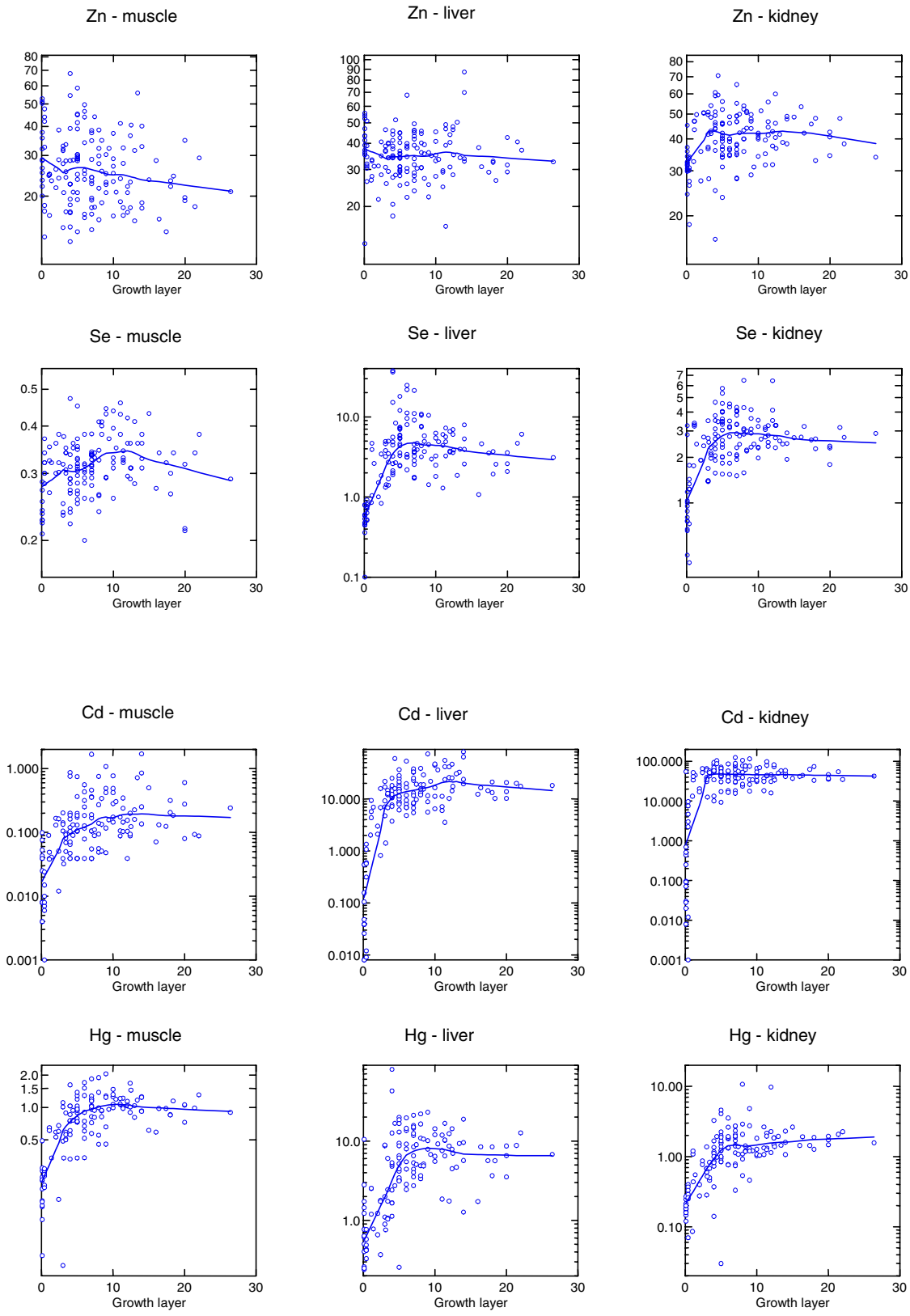

Figure 3.2.1. Relationship between metal concentrations (µg/g w.w.) and age. Lines represent LOWESS smoother.

3.2.2 Correlations

3.2.3 Inter-organ correlation's

Data selection

Metal concentrations had shown to be dependent on both sex and age of the narwhals. Therefore, the data were divided by sex and age (above 3 years old - below or equal 3 years old). Pearson's correlation coefficient between all combinations of metals/stable isotopes and tissues were calculated (Table 3.2.2 and 3.2.3).

Cd

For both sex and both age groups Cd concentrations were highly correlated between tissues. The strongest correlation was found between Cd concentrations in liver and kidney.

Hg

Hg concentrations showed as for Cd to be highly correlated between tissues in both sex and age groups. The inter-organ correlation was lowest for females above 3 years old.

Se

Se concentrations were found to be highest inter-organ correlated in case of liver and kidney for both sex below 3 years old and in case of muscle and kidney for both sex above or equal to 3 years old.

Zn

Zn concentrations were found to be only weakly inter-organ correlated.

3.2.4 Intra-organ correlations

Cd-Zn

Cd-Zn concentrations were highly correlated in liver and kidney of narwhals > 3 years old (Table 3.2.2). For narwhal's • 3 years old Cd-Zn correlations was only high in kidney (Table 3.2.3).

Cd-Hg and Cd-Se

Cd-Hg and Cd-Se concentrations in narwhals • 3 years old were highly correlated in kidney and liver, whereas for narwhals > 3 years old no such correlations were found.

Zn-Hg and Zn-Se

Zn-Hg and Zn-Se concentrations in narwhals • 3 years old were highly correlated in kidney except in case of males. For narwhals > 3 years old no correlation were found.

Hg-Se

Hg-Se concentrations were highly correlated in kidney and liver for both sex and both age groups.

Table 3.2.1. Arithmetic mean (Amean), standard error (SE) geometric mean (Gmean) and relative standard error (rSE) of metal concentrations in muscle, liver and kidney tissues of narwhal samples (µg/g w.w.).

Metal Area	Year	n	Amean±SE (Gmean*/-rse)	n	Amean±SE (Gmean*/-rse)	n	Amean±SE (Gmean*/-rse)
Cd							
Avanersuaq	1984	55	0.155±0.028 (0.081*/1.17)	54	13.3±1.7 (5.30*/1.35)	55	42.6±4.0 (19.8*/1.32)
Avanersuaq	1985	33	0.257±0.060 (0.125*/1.25)	32	11.3±1.8 (4.77*/1.45)	34	37.8±4.3 (15.0*/1.32)
Avanersuaq	1993	4	0.374±0.078 (0.342*/1.30)	1	11.6	1	75.7
Uummannaq	1983	4	0.342±0.093 (0.308*/1.30)	4	18.3±2.9 (17.6*/1.17)	4	38.9±3.6 (38.4*/1.09)
Uummannaq	1993	53	0.163±0.022 (0.110*/1.18)	53	20.9±2.4 (11.8*/1.23)	52	49.4±3.4 (33.3*/1.26)
Balgoni Island	1993	6	0.067±0.020 (0.053*/1.34)	6	6.05±2.32 (1.92*/2.92)	6	29.6±9.1 (8.24*/3.78)
Kitsissuarsuit	1990	3	0.178±0.054 (0.156*/1.48)	2	21.6±9.2 (19.5*/1.57)	2	46.2±4.3 (46.0*/1.10)
Kitsissuarsuit	1994	2	0.041±0.029 (0.029*/2.40)	2	3.81±3.00 (2.36*/2.88)		
Zn							
Avanersuaq	1984	55	29.4±1.4 (27.8*/1.05)	52	36.4±1.0 (35.6*/1.03)	55	39.5±1.2 (38.4*/1.03)
Avanersuaq	1985	33	27.3±2.0 (25.5*/1.07)	32	37.0±1.8 (35.5*/1.05)	34	41.0±1.4 (40.2*/1.03)
Avanersuaq	1993	4	29.3±3.3 (28.7*/1.12)	1	22.2	1	47.7
Uummannaq	1983	4	22.4±2.2 (22.1*/1.10)	4	33.8±2.8 (33.4*/1.08)	4	38.7±3.1 (38.3*/1.08)
Uummannaq	1993	53	27.6±1.5 (25.8*/1.5)	53	35.5±1.2 (34.5*/1.03)	52	43.0±1.3 (41.8*/1.03)
Balgoni Island	1993	6	26.1±3.1 (25.2*/1.12)	6	32.3±4.0 (31.3*/1.11)	6	36.8±3.2 (36.1*/1.09)
Kitsissuarsuit	1990	3	20.4±4.1 (19.7*/1.21)	2	44.2±6.1 (43.8*/1.15)	2	47.5±5.9 (47.1*/1.13)
Kitsissuarsuit	1994	2	28.6±0.2 (28.5*/1.01)	2	28.9±0.6 (28.8*/1.02)		
Hg							
Avanersuaq	1984	55	1.06±0.17 (0.798*/1.11)	52	10.2±1.7 (6.34*/1.16)	54	1.16±0.14 (1.20*/1.13)
Avanersuaq	1985	24	0.534±0.063 (0.402*/1.21)	29	4.35±0.83 (2.37*/1.26)	31	0.954±0.14 (0.64/1.19)
Avanersuaq	1993	4	1.16±0.07 (1.15*/1.07)	1	7.88	1	1.20
Uummannaq	1983	4	0.923±0.081 (0.912*/1.10)	4	5.60±1.24 (5.20*/1.25)	4	1.42±0.27 (1.34*/1.24)
Uummannaq	1993	53	0.896±0.049 (0.798*/1.08)	53	6.49±0.68 (4.04*/1.17)	52	1.40±0.18 (1.07*/1.11)
Balgoni Island	1993	6	0.968±0.323 (0.721*/1.41)	6	8.62±4.42 (3.52*/1.87)	6	3.11±1.66 (1.50*/1.72)
Kitsissuarsuit	1990	3	0.637±0.090 (0.625*/1.15)	2	3.20±1.47 (2.84*/1.64)	2	1.69±0.25 (1.67*/1.16)
Kitsissuarsuit	1994	2	0.350±0.210 (0.280*/2.00)	2	1.41±1.04 (0.95*/2.57)		
Se							
Avanersuaq	1984	51	0.302±0.008 (0.298*/1.02)	52	6.58±1.05 (4.09*/1.16)	54	3.12±0.17 (2.78*/1.08)
Avanersuaq	1985	26	0.290±0.013 (0.283*/1.04)	32	4.21±0.93 (2.35*/1.13)	34	2.10±0.13 (1.94*/1.08)
Avanersuaq	1993	4	0.385±0.033 (0.381*/1.10)	1	4.49	1	2.89
Uummannaq	1983	4	0.263±0.022 (0.260*/1.09)	4	2.83±0.36 (2.75*/1.15)	4	2.12±0.12 (2.10*/1.06)
Uummannaq	1993	53	0.343±0.005 (0.341*/1.01)	53	3.94±0.28 (3.25*/1.10)	52	2.54±0.11 (2.40*/1.05)
Balgoni Island	1993	6	0.348±0.021 (0.345*/1.06)	6	5.25±1.74 (3.55*/1.55)	6	3.33±0.76 (2.79*/1.35)
Kitsissuarsuit	1990	3	0.258±0.020 (0.257*/1.08)	2	1.47±0.40 (1.41*/1.32)	2	2.65±0.08 (2.65*/1.03)
Kitsissuarsuit	1994	2	0.305±0.003 (0.304*/1.01)	2	1.78±0.94 (1.50*/1.81)		

*Table 3.2.2. A correlation matrix of Person's correlation coefficient. Only narwhals > 3 years old are included. * denotes 0.01< p < 0.05, ** denotes p < 0.01.*

Female	Cd-L	Cd-K	Zn-M	Zn-L	Zn-K	Hg-M	Hg-L	Hg-K	Se-M	Se-L	Se-K
Cd-M	**0.61	**0.41	0.19	*0.36	0.09	0.08	0.23	0.14	0.18	0.00	*-0.32
Cd-L		**0.66	0.07	**0.79	0.43	0.18	0.26	0.06	0.01	-0.16	-0.20
Cd-K			0.09	**0.50	**0.72	-0.01	-0.06	0.13	-0.21	*-0.32	-0.01
Zn-M				-0.15	-0.20	0.04	0.00	0.06	-0.11	-0.22	-0.06
Zn-L					**0.55	0.09	0.09	0.03	-0.13	-0.19	-0.13
Zn-K						-0.18	-0.15	0.02	-0.02	-0.20	-0.13
Hg-M							**0.41	0.27	0.11	0.12	0.22
Hg-L								**0.39	0.11	**0.42	0.22
Hg-K									-0.24	0.27	**0.54
Se-M										0.09	**-0.37
Se-L											0.05
Male	Cd-L	Cd-K	Zn-M	Zn-L	Zn-K	Hg-M	Hg-L	Hg-K	Se-M	Se-L	Se-K
Cd-M	**0.38	0.23	0.12	0.09	0.23	0.05	0.14	0.20	0.04	-0.17	0.01
Cd-L		**0.47	0.00	**0.46	*0.30	*0.28	0.25	*0.29	0.03	0.11	0.20
Cd-K			-0.14	0.05	**0.59	-0.24	0.04	-0.24	*-0.27	0.06	*-0.28
Zn-M				0.14	0.02	0.09	-0.05	0.10	0.00	-0.17	0.15
Zn-L					0.08	-0.10	0.02	-0.02	-0.17	0.11	-0.10
Zn-K						*-0.30	*-0.33	-0.21	-0.16	-0.24	-0.23
Hg-M							**0.62	**0.64	**0.41	*0.26	**0.51
Hg-L								**0.52	0.21	**0.53	**0.39
Hg-K									**0.36	*0.27	**0.75
Se-M										-0.03	**0.39
Se-L											0.24

*Table 3.2.3 A correlation matrix of Person's correlation coefficient. Only narwhals • 3 years old are included. * denotes 0.01< p < 0.05, ** denotes p < 0.01.*

Female	Cd-L	Cd-K	Zn-M	Zn-L	Zn-K	Hg-M	Hg-L	Hg-K	Se-M	Se-L	Se-K
Cd-M	*0.53	*0.56	-0.11	-0.07	*0.53	0.36	**0.63	**0.61	0.03	*0.61	*0.53
Cd-L		**0.84	-0.27	-0.42	*0.58	*0.54	*0.53	**0.80	*0.63	**0.84	**0.72
Cd-K			-0.28	-0.33	**0.69	*0.52	**0.65	**0.79	0.35	**0.74	**0.88
Zn-M				0.13	-0.15	0.15	0.16	0.05	0.09	-0.06	-0.04
Zn-L					0.22	-0.31	0.09	-0.12	-0.46	-0.24	-0.34
Zn-K						0.45	**0.69	**0.70	0.10	0.61	**0.68
Hg-M							**0.72	**0.76	0.39	**0.70	**0.78
Hg-L								**0.80	0.19	**0.79	**0.79
Hg-K									**0.54	**0.79	**0.89
Se-M										*0.54	0.25
Se-L											**0.81
Male	Cd-L	Cd-K	Zn-M	Zn-L	Zn-K	Hg-M	Hg-L	Hg-K	Se-M	Se-L	Se-K
Cd-M	**0.63	**0.65	-0.05	-0.17	**0.76	**0.63	*0.50	*0.50	-0.09	**0.55	**0.50
Cd-L		**0.93	-0.02	*-0.44	**0.73	**0.82	**0.60	**0.74	0.42	**0.75	**0.74
Cd-K			-0.10	*-0.53	**0.73	**0.82	**0.62	**0.74	0.29	**0.76	**0.81
Zn-M				0.19	-0.16	0.00	-0.13	-0.01	-0.14	-0.01	0.09
Zn-L					-0.08	-0.11	0.07	-0.07	-0.34	-0.18	0.32
Zn-K						**0.67	**0.63	**0.80	0.03	0.71	0.67
Hg-M							**0.84	**0.86	0.43	**0.87	**0.72
Hg-L								**0.71	0.16	**0.79	**0.56
Hg-K									0.31	**0.82	**0.71
Se-M										0.26	0.34
Se-L											**0.72

3.2.5 Geographical differences

Other factors

The main aim of this study was to detect whether there were differences in metal concentrations between narwhals from different locations. However, preliminary analysis have showed that factors such as sex and sampling year may also influence the metal concentrations found in the narwhals. Therefore, the data has to be treated by groups not only by location but also by sex and year of sampling.

Data selection

Only groups (location-sex-sampling year combination) with 3 or more individuals are included in this geographical comparison. Furthermore, as a consequence of the age influence on all metal concentrations except Zn only narwhals older than 3 years old are included (except for Zn).

Data treatment

Table 3.2.4 and Figure 3.2.2 shows arithmetic mean of metal concentrations and standard error. Table 3.2.5 shows results of two-way ANOVA's including the effects of location (loc), sex and interaction between location and sex (loc*sex) performed on each metal and tissue combination

Cd

In all tissue (muscle, liver and kidney) the Cd concentrations were significantly higher in females than in males. In some of the tissues a

consistent difference between Avanersuaq and Uummannaq was found. In muscle tissue there was significant difference between the samples in 1993 and 1984 from Avanersuaq. However, the sample in 1985 was not different from the two others. In the sample from Uummannaq 1993, Cd concentrations especially in females were higher than in the other samples.

Hg

In all tissue (muscle, liver and kidney) Hg concentrations were higher in females than in males. However, only in liver this difference was significant. The three tissues showed a consistent difference between areas and years. However, the results (Avanarsuaq 1984 were highest, Uumannaq 1993 and 1983 were intermediate and Avanersuaq 1985 were lowest) indicate that year to year variation was more important in explaining differences than regions.

Se

In all tissue (muscle, liver and kidney) Se concentrations as for Hg were higher in females than in males. However, only in liver this difference was significant. In muscle and liver tissue no consistant difference between Avanersuaq and Uummannaq was found. In kidney Se concentrations in 1993 in narwhals from Avanersuaq was significantly higher than the other samples.

***Table 3.2.4**. Arithmetic mean (AM µg/g w.w.) and standard error (SE) of metals concentrations in muscle, liver and kidney tissues of narwhal samples divided by sex. Only narwhals above 3 years old and sample size • 3 are included.*

Metal		n		Muscle (AM±SE)		Muscle (AM±SE)		Muscle (AM±SE)	
Area	Year	F	M	F	M	F	M	F	M
Cd									
Avanersuaq	1984	25	11	0.217±0.111	0.092±0.051	19.5±5.9	10.4±3.0	57.2±10.3	33.9±20.5
Avanersuaq	1985	5	18	0.487±0.429[1]	0.310±0.205	23.6±16.0	11.2±3.3[2]	64.3±25.7	39.7±9.3
Avanersuaq	1993	3	-	0.445±0.143	-	-	-	-	-
Uummannaq	1983	-	4	-	0.342±0.258	-	18.7±8.0	-	38.9±10.1
Uummannaq	1993	14	28	0.323±0.130	0.128±0.022	39.9±11.3	17.5±3.2	69.6±10.9	49.6±6.4[3]
Hg									
Avanersuaq	1984	25	11	1.07±0.12	1.82±1.79	11.4±3.9[4]	16.3±16.1[5]	1.81±0.3	2.03±0.80
Avanersuaq	1985	5	12	0.662±0.382[1]	0.707±0.132	10.2±5.0	4.44±2.21	2.02±0.84	1.08±0.37[7]
Avanersuaq	1993	3	-	1.19±0.29	-	-	-	-	-
Uummannaq	1983	-	4	-	0.923±0.225	-	5.60±3.43	-	1.42±0.74
Uummannaq	1993	14	28	30.3±5.7	0.974±0.086	10.8±3.2	6.32±1.26	1.31±0.21	1.78±0.67[3]
Zn									
Avanersuaq	1984	35	18	30.0±4.0	28.7±4.0	36.8±2.7[8]	35.5±3.7[2]	42.3±2.9	34.3±4.3
Avanersuaq	1985	6	23	23.4±5.2	29.5±5.6	38.2±11.9[9]	37.8±4.1	39.2±5.5[9]	42.1±3.6
Avanersuaq	1993	3	-	28.5±19.4	-	-	-	-	-
Uummannaq	1983	-	4	-	22.4±7.1	-	33.8±8.9	-	38.7±10.0
Uummannaq	1993	18	35	30.3±5.7	26.3±3.5	40.0±5.4	33.2±2.2	43.6±5.4	42.6±3.1[8]
Se									
Avanersuaq	1984	25	11	0.314±0.018	0.321±0.046	7.83±3.06[11]	9.87±8.36[5]	3.60±0.36	3.65±0.72[5]
Avanersuaq	1985	3	17	0.296±0.103	0.300±0.038	12.0±11.5[12]	3.90±1.38[2]	2.30±0.66	2.40±0.32[13]
Avanersuaq	1993	3	-	0.377±0.192	-	-	-	-	-
Uummannaq	1983	-	4	-	0.263±0.069	-	2.83±1.15	-	2.12±0.38
Uummannaq	1993	14	28	0.353±0.016	0.343±0.016	5.91±0.93	3.88±0.53	2.40±0.24	2.92±0.31[3]

[1]n=4, [2]n=16, [3]n=27, [4]n=23, [5]n=10, [6]n=14, [7]n=15, [8]n=34, [9]n=7, [10]n=21, [11]n=24, [12]n=5, [13]n=18

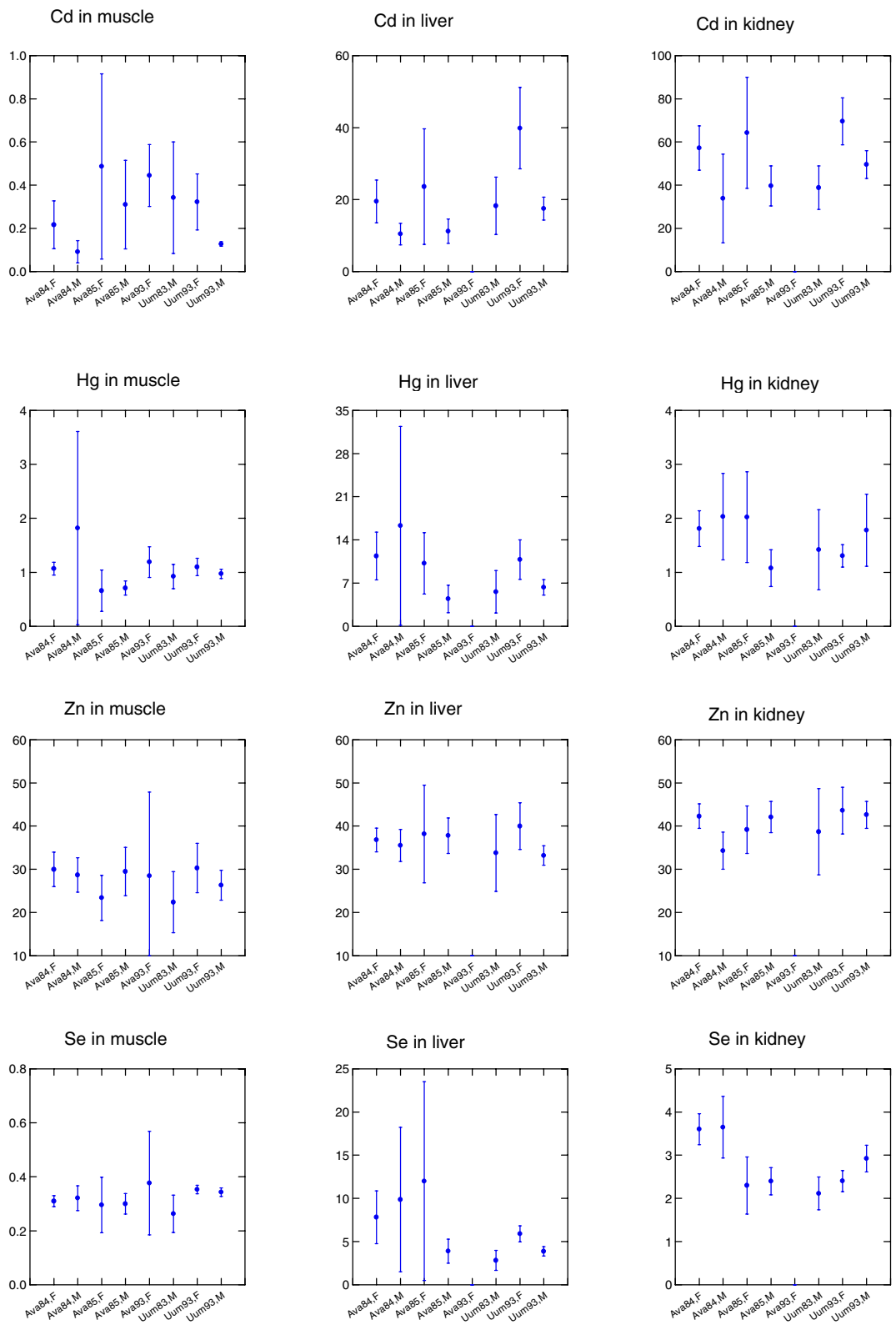

Figure 3.2.2. Mean (µg/g w.w.±standard error) of metal concentrations in narwhals divided by area, year and sex.

*Table **3.2.5**. Results of two-way ANOVA's including the effects of location (loc), sex and interaction between location and sex (loc*sex) performed on each metal and tissue combination.* denotes p < 0.05. Locations or sex underlined were not significant different at the 5% level tested by Tukey's studentized range test. Only narwhals >3 years old are included.*

		loc	sex	loc*sex	Location					Sex	
Cd	Muscle	*	*	-	A93	U83	A85	U93	A84	F	M
	Liver	*	*	-	U93	U83	A84	A85		F	M
	Kidney	*	*	-	U93	A84	A85	U83		F	M
Zn	Muscle	-	-	-	A93	A84	A85	U93	U83	F	M
	Liver	-	-	-	A85	A84	U93	U83		F	M
	Kidney	-	-	*							
Hg	Muscle	*	-	-	A93	A84	U93	U83	A85	F	M
	Liver	*	*	-	A84	U93	U83	A85		F	M
	Kidney	-	-	-	A84	U93	A83	A85		F	M
Se	Muscle	*	-	-	A93	U93	A84	A85	U83	F	M
	Liver	*	*	-	A84	U93	A85	U83		F	M
	Kidney	*	-	-	A84	U93	A85	U83		F	M

In general, several differences in mean metal concentrations between localities (locality and year combinations) were found except for Zn. However, in most cases the year-to-year variation exceeded the geographical differences. Therefore the metal analyses do not indicate that the narwhal populations from Avanersuaq and Uummannaq are different.

3.2.6 Comparison with other Arctic regions

Data on metal concentration in narwhal tissues from several locations in Arctic Canada is summarized by Dietz *et al.* (1998). In general, the levels of Cd, Hg and Se found in the Greenland samples are within the range found in Arctic Canada (Wagemann *et al.* 1983, 1996).

3.3 Persistent Organic Pollutants (POP)

In total, POP concentrations in 94 blubber samples of narwhals were analysed. The statistical analysis of differences between locations, relationship with age, correlation's were conducted on sum of PCB congeners (28, 31, 52, 101, 105, 118, 138, 163, 153, 156 and 180), HCB, sum of HCHs (α-HCH, β-HCH and γ-HCH), sum of DDTs (p,p'-DDE, p,p'-DDD and p,p'-DDT), trans-nonachlor, total toxaphene and sum of the toxaphene congeners 26, 32, 50 and 62 (CHBs). Table 3.3.1 and 3.3.2 shows arithmetic means (standard errors) and geometric means (relative standard errors), respectively. The arithmetic means are also shown in Figure 3.3.1. It is well established that POP concentrations depend on age and sex (e.g. AMAP 1998). It is therefore necessary to

take these factors into account when comparing POP levels between locations and/or years.

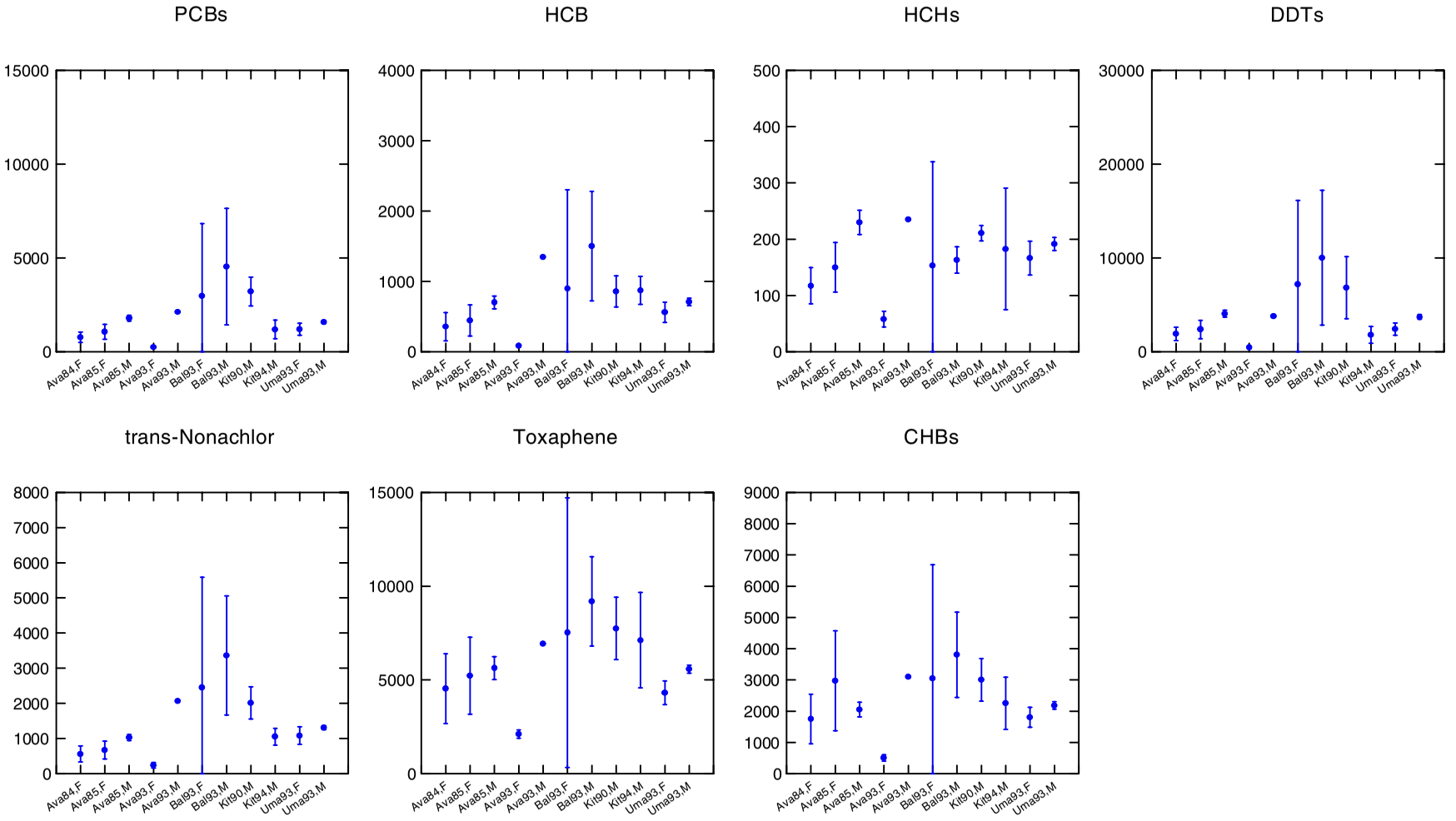

Figure 3.3.1. Mean (±standard error) of POP in narwhal blubber (µg/kg l.w.)

Table 3.3.1. Arithmetic mean (µg/kg lipid weight/l.w.) and standard error of POP in narwhals blubber by location, sex and age group.

Location, year	Sex	Age group	N	PCBs	HCBs	HCHs	DDTs	trans-nonachlor	Toxaphenes	CHBs
Ava., 1984	F	Juvenile	1	1287	814	157	3227	1029	8925	3245
		Maturing	1	1010	383	148	2573	717	4561	2488
		Adult	2	388 (149)	112 (76)	82 (61)	915 (146)	253 (42)	2334 (348)	638 (52)
Ava., 1985	-	Yearling	1	356	250	124	569	250	2600	769
		Juvenile	1	2222	633	233	6938	2088	5354	1520
		Adult	2	2055(749)	870(184)	246(6)	4725(2114)	1100(0)	6200(424)	2230(147)
	F	Yearling	1	2563	1349	306	6276	1561	13978	8637
		Maturing	1	2135	1076	293	4908	1552	10682	8666
		Adult	5	549(493)	136(85)	90(37)	1093(970)	319(179)	2391(879)	705(285)
	M	Yearling	2	767(745)	393(297)	191(99)	1705(1720)	473(358)	2428(637)	1037(696)
		Juvenile	6	1766(442)	963(280)	279(69)	3696(918)	1186(196)	7064(2436)	2316(807)
		Maturing	1	2365	625	193	5403	1121	5326	2714
		Adult	6	2059(350)	516(86)	189(44)	4850(715)	1005(230)	5100(783)	1944(818)
Ava., 1993	F	Adult	3	239(77)	80(20)	55(16)	436(147)	233(101)	2033(321)	487(137)
	M	Adult	1	2114	1300	227	3650	2000	6700	2996
Uum., 1993	F	Yearling	2	2812(2511)	1498(1002)	382(96)	5269(4728)	2388(1794)	8011(1445)	3189(779)
		Juvenile	4	2061(1116)	861(309)	233 (99)	4308(2646)	1728(795)	5981(1564)	2857(810)
		Adult	11	591(586)	270(303)	99(53)	1161(1220)	588(539)	2930(1746)	1130(990)
Uum., 1993	M	Yearling	4	1031(268)	778(91)	314(25)	1856(544)	1000(179)	6786(1055)	3025(971)
		Juvenile	9	1496(490)	867(422)	194(48)	2903(1238)	1306(208)	5080(803)	1978(485)
		Adult	19	1732(410)	573(176)	157(40)	4219(1071)	1295(309)	5205(1130)	1987(588)
		-	1	1715	610	113	3700	1100	5400	1917
Bal., 1993	F	Juvenile	1	5076	1600	244	11600	4000	11000	4840
		Adult	1	875	130	51	2240	720	3500	1045
	M	Yearling	1	756	390	166	1730	1100	4500	1336
		Juvenile	2	6946(7319)	2200(1556)	177(18)	15035(16921)	4600(3818)	11400(3677)	5015(2437)
		Adult	1	3494	1000	104	7080	2700	8000	3287
Kit., 1990	M	Adult	2	3210(589)	830(170)	204(11)	6605(2496)	1950(354)	7500(1273)	2909(523)
Kit., 1994	M	Juvenile	2	1187(383)	835(106)	178(88)	1715(587)	1010(127)	6800(1556)	2153(516)

Table 3.3.2. *Geometric mean (µg/kg l.w.) and relative standard error (in brackets) of POP in narwhal blubber by location, sex and age group.*

Location, year	Sex	Age group	N	PCBs	HCBs	HCHs	DDTs	trans-nonachlor	Toxa-phenes	CHBs
Ava., 1984	F	Juvenle	1	1287	814	157	3227	1029	8925	3245
		Maturing	1	1010	383	148	1273	717	4561	2488
		Adult	2	374(1.48)	98(2.10)	70(2.29)	909(1.17)	251(1.18)	2321(1.16)	637(1.08)
Ava., 1985	-	Yearling	1	356	250	124	569	250	2600	769
		Juvenile	1	2222	633	233	6938	2088	5354	1520
		Adult	2	1987(1.44)	860(1.24)	246(1.02)	4482(1.59)	1100(1.00)	6193(1.07)	2228(1.07)
	F	Yearlng	1	2563	1349	306	6276	1561	13978	8637
		Maturing	1	2135	1076	293	4908	1552	10682	8666
		Adult	5	436(1.99)	117(1.83)	83(1.59)	871(1.98)	290(1.57)	2283(1.39)	667(1.42)
	M	Yearling	2	558(3.29)	333(2.32)	177(1.72)	1195(3.54)	399(2.33)	2386(1.30)	913(2.07)
		Juvenile	6	1722(1.28)	925(1.40)	272(1.28)	3600(1.29)	1174(1.17)	6753(1.38)	2216(1.37)
		Maturing	1	2365	625	193	5403	1121	5326	2714
		Adult	6	2037(1.17)	510(1.19)	185(1.25)	4802(1.17)	986(1.24)	5054(1.15)	1830(1.44)
Ava., 1993	F	Adult	3	231(1.36)	78(1.28)	54(1.33)	419(1.41)	219(1.56)	2017(1.17)	476(1.30)
	M	Adult	1	2114	1300	227	3650	2000	6700	2996
Uum., 1993	M	Yearling	2	2181(2.86)	1320(2.07)	376(1.29)	4072(2.88)	2023(2.31	7945(1.20)	3141(1.28)
		Juvenile	4	1822(1.79)	825(1.39)	213(1.70)	3648(1.98)	1588(1.61)	5835(1.29)	2761(1.37)
		Adult	11	399(2.47)	160(2.83)	87(1.71)	774(2.47)	423(2.27)	2560(1.68)	863(2.08)
Uum., 1993	M	Yearling	4	1005(1.30)	774(1.13)	313(1.08)	1793(1.36)	987(1.21)	6723(1.17)	2890(1.44)
	F	Juvenile	9	1429(1.37)	784(1.61)	188(1.30)	2696(1.49)	1290(1.19)	5022(1.18)	1923(1.29)
		Adult	19	1647(1.48)	524(1.71)	151(1.34)	3976(1.54)	1241(1.41)	5059(1.30)	1903(1.37)
		-	1	1715	610	113	3700	1100	5400	1917
Bal., 1993	F	Juvenile	1	5076	1600	244	11600	4000	11000	4840
		Adult	1	875	130	51	2240	720	3500	1045
	M	Yearling	1	756	390	166	1730	1100	4500	1336
		Juvenile	2	4632(3.90)	1905(2.17)	176(1.11)	9104(4.65)	3724(2.59)	11100(1.39)	4709(1.66)
		Adult	1	3494	1000	104	7080	2700	8000	3287
Kit., 1990	M	Adult	2	3182(1.20)	821(1.22)	204(1.06)	6365(1.47)	1934(1.20)	7446(1.19)	2885(1.20)
Kit., 1994	M	Juvenile	2	1155(1.39)	832(1.14)	166(1.68)	1664(1.42)	1006(1.13)	6710(1.26)	2122(1.27)

3.3.1 Influence of age on POP concentrations

The relationship between POP concentration and age of narwhals is shown in Figure 3.3.2. The aim of the figure is only to present a general pattern of the relationships illustrated by use of the LOWESS smoother. Therefore, no attempt was made in this figure to elucidate the dependency of location the whales derived from. Females and males showed quite different relationships between POP concentrations and age.

Females

All POP concentrations in females showed the same pattern with a decreasing trend from the yearlings to an age of about 8 to 10 years. Animals between 10 to 15 years showed an increasing POP concentration. The lower levels of POP in adult females compared to younger females and adult males are common in cetaceans and pinnipeds because of elimination of these lipophilic compounds via lactation (e.g. Addison and Smith 1974). The increase from 10 years to 15 years was therefore unexpected. However, high levels in only 3-4 individuals created this pattern.

Males

PCBs, DDTs and trans-nonachlor in the analysed male narwhals showed an increase in the first few years, where after no increase or a small gradual increase with increasing age were observed. HCHs, toxaphene, CHBs (and to lesser extent HCB) showed a decrease in the first few years of life followed by a gradual increase with increasing age.

3.3.2 Correlations

The concentration of all the analysed POPs were highly intercorrelated (Table 3.3.3).

*Table **3.3.3**. Correlation matrix of Pearson's correlation coefficient (POP concentrations log-transformed). ** denotes p<0.01.*

	HCB	HCHs	DDTs	trans-nonachlor	toxa-phenes	CHBs
PCBs	0.88**	0.67**	0.98**	0.95**	0.86**	0.84**
HCB		0.86**	0.83**	0.91**	0.92**	0.89**
HCHs			0.63**	0.68**	0.76**	0.74**
DDTs				0.93**	0.83**	0.81**
trans-nonachlor					0.88**	0.86**
toxaphenes						0.95**

3.3.3 Geographical differences

PCBs

The PCBs showed a significant dependence on age with a significantly different relationship for the two sexes. In females the PCBs decreased significantly with increasing age whereas in males the

PCBs increased slightly but not significantly with age. No significant differences in age adjusted mean PCBs between localities was found (Table 3.3.4). The estimated PCBs concentration in a 7 years old male narwhal is shown in Table 3.3.5.

HCB

HCB also showed a significant dependence on age with significantly different relationship for the two sexes. In females, HCB decreased significantly with increasing age. In males HCB also decrease with age, however, only slightly and not significantly. No significant difference in age adjusted mean HCB between localities was found (Table 3.3.4). The estimated HCB concentration in a 7 years old male narwhal is shown in Table 3.3.5.

HCHs

HCHs showed similar results of the statistical analyses as HCB with an increase with age dependent on sex. In both sexes the HCHs decreased significantly with age, however, the decrease was more pronounced in females than in males. No significant differences in age adjusted mean HCHs between localities was found (Table 3.3.4). The estimated HCH concentration in a 7 years old male narwhal is shown in Table 3.3.5.

DDTs

The DDTs showed a significant decrease with age in females and a significant increase in males. The age adjusted mean DDTs concentration showed a significant higher concentration in narwhals from Balgoni Island, 1993 than all other locations except Kitsissuarsuit, 1990, which did not differ from any of the other locations (Table 3.3.4). The estimated DDTs concentration in a 7 years old male narwhal is shown in Table 3.3.5.

Trans-nonachlor

Trans-nonachlor showed a significant decrease with age in females and a slightly non-significant increase in males. The age adjusted mean trans-nonachlor concentration showed a significant higher concentration in narwhals from Balgoni Island, 1993 compared to all other locations except Kitsissuarsuit, 1990, which did not differ from any locations (Table 3.3.4). The estimated trans-nonachlor concentration in a 7 years old male narwhal is shown in Table 3.3.5.

Toxaphene

Toxaphene concentrations decrease significantly with age in females and with no changes with age in males. No significant difference in age adjusted mean toxaphene between localities was found. The estimated toxaphene concentration in a 7 years old male narwhal is shown in Table 3.3.5.

CHBs

CHBs concentrations decrease significantly with age in females and with no changes with age in males. No significant difference in age adjusted mean CHBs between localities was found (Table 3.3.4). The estimated CHBs concentration in a 7 years old male narwhal is shown in Table 3.3.5.

Summary

In general, the results from the statistical analysis were very similar for the different POP groups. Only few differences in mean levels between locations were found, whereas the dependence of age different for the two sex's were very pronounced. However, for all POP groups the concentration was highest at locations as Balgoni Island, 1993 and Kitsissuarsuit, 1990 and lowest in locations as Avanersuaq 1984 and 1993 (Table 3.3.4). One reason for the lack of observed dif-

ferences in POP levels between locations may be that the variability between individuals was very high and the numbers of individuals in each location-sex combination are low. This results in a low power of the statistical analysis to detects significant differences among locations.

*Table **3.3.4.** Results of the least square mean test of difference in age adjusted mean POP concentrations between locations. Locations are in decreasing order and the underlining indicates no significant difference.*

PCBs	Bal93	Kit90	Ava85	Uma93	Kit94	Ava84	Ava93
HCB	Kit90	Bal93	Kit94	Uma93	Ava85	Ava84	Ava93
HCHs	Kit90	Uma93	Ava85	Kit94	Ava84	Bal93	Ava93
DDTs	Bal93	Kit90	Ava85	Ava84	Uma93	Kit94	Ava93
trans-nonachlor	Bal93	Kit90	Uma93	Kit94	Ava85	Ava84	Ava93
Toxaphenes	Bal93	Kit90	Kit94	Uma93	Ava85	Ava84	Ava93
CHBs	Bal93	Kit90	Kit94	Uma93	Ava85	Ava84	Ava93

Table 3.3.5. Estimated mean and standard deviation of POP concentrations (µg/kg l.w.) in a 7 year old male narwhale.

Location, year	PCBs	HCB	HCHs	DDTs	trans-nonachlor	toxaphene	CHBs
Avanersuaq, 1984	1518	637	158	3982	906	5494	2080
	(1182)	(529)	(107)	(3148)	(664)	(3669)	(1516)
Avanersuaq, 1985	2113	804	219	4840	1128	5678	2299
	(1737)	(715)	(151)	(4054)	(862)	(3872)	(1744)
Avanersuaq, 1993	1044	556	148	1935	861	4648	1504
	(801)	(454)	(99)	(1508)	(625)	(3088)	(1086)
Uummannaq, 1993	1899	997	223	3986	1542	5946	2526
	(1499)	(842)	(152)	(3197)	(1142)	(3992)	(1859)
Balgoni Island, 1993	4073	1288	157	9419	3142	8784	3585
	(3246)	(1059)	(105)	(7385)	(2289)	(5849)	(2598)
Kitsissuarsuit, 1990	3365	1317	274	6116	2171	8201	3450
	(2610)	(1089)	(185)	(4818)	(1587)	(5469)	(2507)
Kitsissuarsuit, 1994	1633	1032	166	2537	1256	7361	2539
	(1262)	(849)	(112)	(1990)	(916)	(4902)	(1840)

3.3.4 PCB congener pattern

The relative concentrations of PCB congeners have shown to be useful in discrimination of marine mammals from different areas (e.g. Cleeman et al. 2000, Muir et al. 2000). Although the sum of PCB congeners does not differ among locations it may be that the relative pattern of congeners differ significantly.

Data

The PCB congeners included are CB28, CB31, CB52, CB101, CB 118, CB138, CB153, CB156 and CB180. In several cases the PCB congener (especially CB-28, 31 and 156) were below detection limit and the half of the value of detection limit were used in the analysis. This may have some influence on the statistical analysis and limit the conclusions that can be drawn.

Results

Five principal components had eigen-values above 1. The three first components explained 68% of the total variation (28.0%, 26.0% and 13.8%, respectively). Table 3.3.6 shows the congener correlation with the three principal components.

Table 3.3.6. Congener correlations with the first three principal components together with the cummulative part of the total variance explained

PCBs	No,Cl	Chloro-substitute	PC1	PC2	PC3
CB-28	3	2,4,4'	-0.02	0.93	-0.08
CB-31	3	2,4',5	-0.03	0.79	0.35
CB-52	4	2,2',5,5'	-0.69	-0.54	-0.19
CB-101	5	2,2',4,5,5'	-0.63	-0.65	-0.08
CB-105	5	2,3,3',4,4'	-0.03	0.09	0.93
CB-118	5	2,3',4,4'5	-0.36	-0.24	0.05
CB-138	6	2,2'3,4,4',5'	0.85	-0.11	0.14
CB-153	6	2,2'4,4'5,5'	0.80	-0.13	-0.42
CB-156	6	2,3,3',4,4',5	0.12	0.26	0.27
CB-180	7	2,2'3,4,4'5,5'	0.65	0.49	-0.27

PC1

PC1 describes differences between the higher chlorinated (Cl atoms ≥6) and the congeners CB-52 and CB-101. CB-52 and CB-101 have both *meta* and *para* vicinal H atoms and two *ortho* Cl atoms. No significant difference in mean PC1 was found (ANOVA, $p=0.11$).

PC2

PC2 describes the differences between the two low chlorinated congeners CB-28 and CB-31 l atoms ≥6) and again the congeners CB-52 and CB-101. The mean PC2 differed significantly among locations (ANOVA, $p<0.01$).

PC3

The most important PCB congener for PC3 is CB-105. The mean PC3 differed significantly among location (ANOVA, $p<0.001$) with the significant highest mean values in Avanersuaq 1984 and 1985 narwhals (Tukey's studentized range test, $p<0.05$).

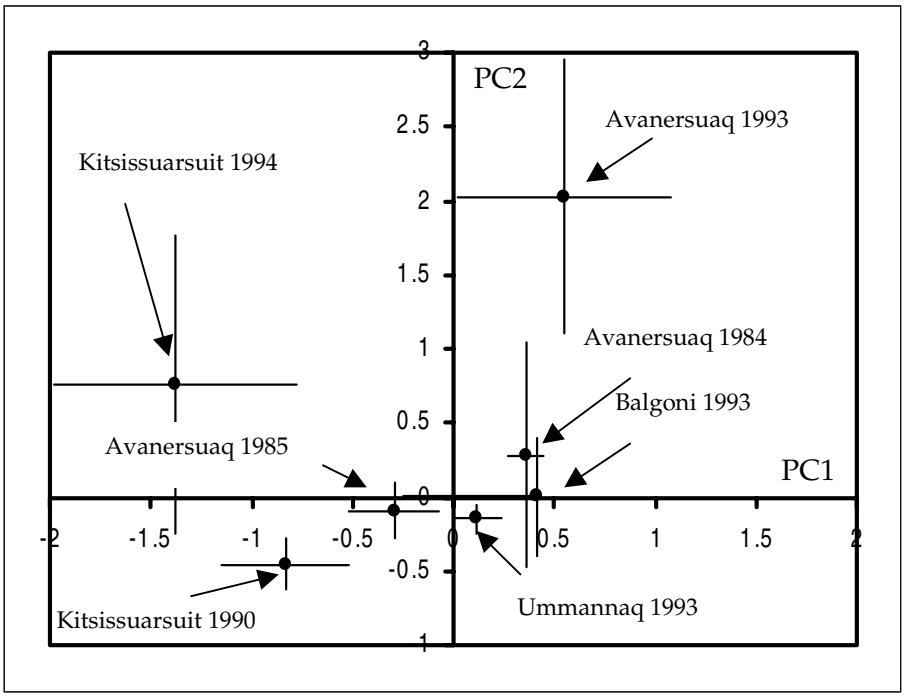

Figure 3.3.3. *PCA analysis on PCB congeners in narwhals. Plot of mean and standard errors of PC1 versus PC2.*

Summary

The pattern of the PCBs congeners showed differences among locations. Figure 3.3.3 indicates some isolation of the Avanersuaq 1993 and Kitsissuarsuit 1994 narwhals from the other samples of narwhals. Avanersuaq 1993 and Kitsissuarsuit 1994 are mainly separated from each other by the value of PC1 meaning that the concentrations of higher chlorinated congeners and those of PC-101 and CB-105 are lower in narwhals from Avanersuaq 1984 than in narwhals from Kitsissuarsuit. However, as mentioned before, the results of the PCA analysis was disturbed by many results below detection limits especially for CB-28, 31 and 156. Thus, although differences in PCB congene pattern between localities were observed, year-to-year variations are more pronounced. Therefore PCB congene pattern do not indicate that the narwhal populations from Avanersuaq and Uummannaq are different.

3.3.5 Comparison with other Arctic regions

In monodontiids (beluga and narwhal) toxaphene is reported to be the most dominant POP (Muir et al. 1999). This was supported by this study, where the mean values normalised to a 7 years old male narwhal ranged between 5.2 µg/g l.w. to 10 µg/g l.w. Beck (1994) cited in Muir et al. 1999 reports on levels in 8 males from Lancaster Sound sampled in 1991. PCBs seem to be at a little lower or at the same level in the Greenland samples than in the Canadian samples, whereas HCHs and DDTs seem to be higher.

3.4 Stable isotope analysis

$\delta^{15}N$

Measurement of the concentrations of the naturally occurring stable isotopes of nitrogen (^{15}N,^{14}N) can be used to estimate the trophic level of a species. The reason is that the abundance of $\delta^{15}N$ in the tissues of consumers is typically enriched over that in their prey owing to the preferential excretion of the lighter ^{14}N during protein transamination and deamination (Hobson and Welch 1995). As a consequence, the ratio $^{15}N/^{14}N$ will increase with trophic level. The ratio $^{15}N/^{14}N$ expressed as per mille is denoted $\delta^{15}N$.

$\delta^{13}C$

Carbon is also occurring naturally in a stable isotope (^{13}C). However, the trophical fractionation of $\delta^{13}C$ is typically small compared to $\delta^{15}N$ (Hobson and Welch 1995). $\delta^{13}C$ is more an indicator of sources of primary production rather than trophic position. However, the measurement of both carbon and nitrogen isotopes can often yield greater information on feeding differences between organisms than the measurement of one of the isotopes (Hobson and Welch 1995)

Table 3.4.1 shows descriptive statistic of stable isotopes both for the total material and for narwhals > 3 years and sample size ≥3 (in order to compare with metal concentrations in Table 3.2.4).

*Table **3.4.1**. Arithmetic mean (AM) standard error (S.E.) of stable isotopes in narwhals*

$\delta^{15}N$		All samples			Narwhals > 3 years and sample size ≥3					
					Females			Males		
Locality	Year	N	AM	S.E.	N	AM	S.E.	N	AM	S.E.
Avanersuaq	1984	50	16.02	0.13	24	15.62	0.23	11	15.87	0.37
	1985	33	16.03	0.18	5	15.70	0.74	17	15.59	0.18
	1993	4	16.20	0.56	3	16.24	2.49			
Uummannaq	1993	53	16.59	0.09	14	16.41	0.19	28	16.43	0.23
Balgoni Island	1993	6	17.21	0.57						
Kitsissuarsuit	1990	3	15.88	0.37						
	1994	2	16.29	0.61						
$\delta^{13}C$		All samples			Narwhals > 3 years and sample size ≥3					
					Females			Males		
Locality	Year	N	AM	S.E.	N	AM	S.E.	N	AM	S.E.
Avanersuaq	1984	50	-18.35	0.03	24	-18.39	0.06	11	-18.31	0.20
	1985	33	-18.50	0.03	5	-18.57	0.21	17	-18.47	0.10
	1993	4	-18.31	0.11	3	-18.30	0.50			
Uummannaq	1993	53	-18.31	0.05	14	-18.51	0.13	28	-18.17	0.14
Balgoni Island	1993	6	-18.53	0.15						
Kitsissuarsuit	1990	3	-17.93	0.11						
	1994	2	-18.14	0.08						

3.4.1 Influence of age on stable isotope

The relationship between stable isotope and age of narwhal is shown in Figure 3.4.1. The aim of Figure 3.4.1 is only to present a general pattern of the relationships. Therefore, no attention has been paid in this figure to the location they derive from or the sex of the narwhal.

$\delta^{15}N$

δ^{15}N showed a decreasing value during the first year of the narwhal life (Figure 3.4.1). Thereafter the δ^{15}N value was rather constant. The reason of this is that the narwhal in the first year live of mother's milk and as a consequence has a δ^{15}N value corresponding to be living at a very high tropic level (i.e. predation on the mother).

$\delta^{13}C$

The pattern of δ^{13}C during life was different from that of δ^{15}N as no clear changes was observed (Figure 3.4.1).

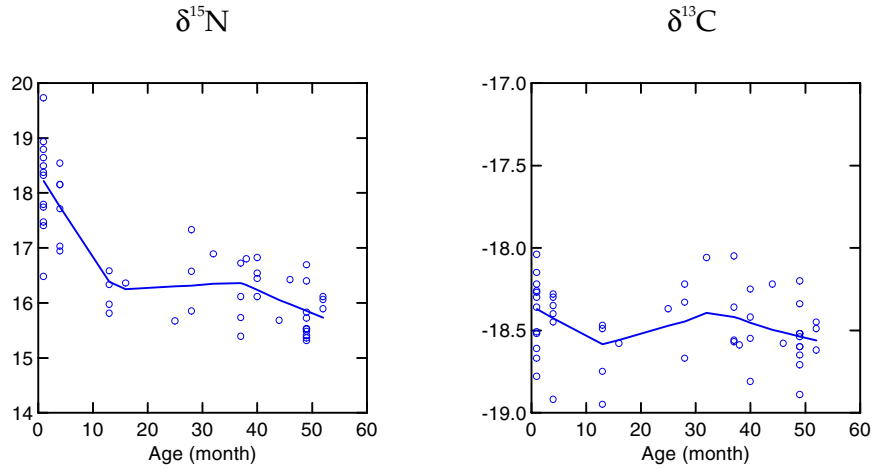

Figure 3.4.1. *Relationship between stable isotopes and age. Lines represent LOW-ESS smoother.*

3.4.2 Geographical differences

Data selection

Only groups (location-sex-sampling year combination) with 3 or more individuals were included in this geographical comparison. Furthermore, as a consequence of the age influence on stable isotope values only narwhals older than 3 years old were included.

Results

Table 3.4.1 and Fig. 3.4.2 shows arithmetic mean metal concentrations and standard error. The results of the statistical analyses are shown in Table 3.4.2.

*Table 3.4.2. Results of two-way ANOVA's including the effects of location (loc), sex and interaction between loc and sex (loc*sex) performed on stable isotopes (SI).* denotes p > 0.05. Locations or sex underlined were not significant different at the 5% level tested by Tukey's studentized range test. Only narwhal >3 years old were included.*

SI	loc	sex	loc*sex	Locations			Sex	
$\delta^{15}N$	*		-	U93	A84	A85	F	M
$\delta^{13}C$	*	*	-	U93	A84	A85	M	F

$\delta^{15}N$

$\delta^{15}N$ showed no significant difference in mean values between sex, but significantly higher mean $\delta^{15}N$ value in narwhal from Uummannaq than in narwhals from Avanersuaq.

$\delta^{13}C$ $\delta^{13}C$ mean values were significantly higher in males than in females. No clear difference was found between narwhals from Avanersuaq and Uummannaq.

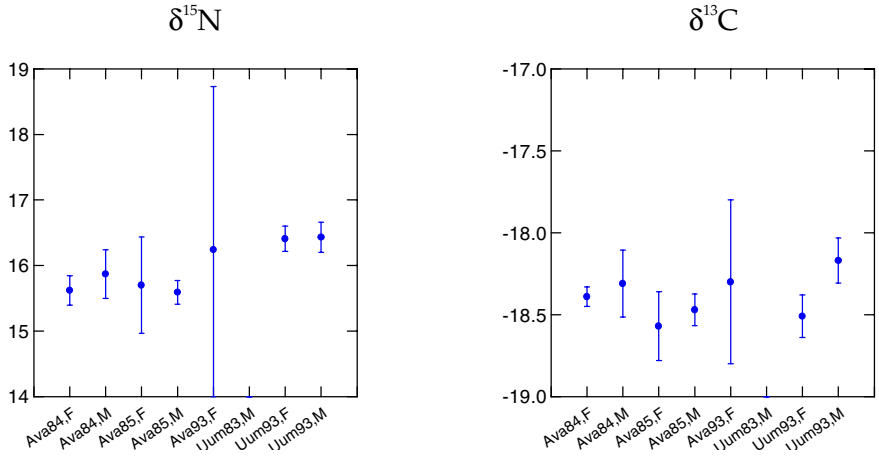

Figure 3.4.2. Mean (± standard deviation) of stable isotopes in narwhals.

3.4.3 Correlation's with metal and POP concentrations

Metals

For narwhals • 3 years old $\delta^{15}N$ was significantly (at 5 % level) negatively correlated with Cd, Zn, Hg and Se concentrations in kidney and Cd, Hg and Se concentrations in liver for both sex. In addition, $\delta^{15}N$ was significantly negatively correlated with Se concentrations in muscle of males and with Cd concentrations in muscle of females (Table 3.4.3). This pattern reflects the decrease of $\delta^{15}N$ simultaneously with the accumulation metals with age.

For narwhals > 3 years old only few significant correlations between metal concentrations and $\delta^{15}N$ were found (Table 3.4.3). In case of females, $\delta^{15}N$ was significantly correlated with Cd and Se concentrations in muscle, and in case of males $\delta^{15}N$ were significantly correlated with Hg and Se concentrations in liver and kidney. The low degree of correlations between $\delta^{15}N$ and heavy metals does not support a general relationship between trophic level and heavy metal concentrations in the individual narwhal.

$\delta^{13}C$ was found only to be significant correlated with metal concentrations in a few occasions (Table 3.2.3). For narwhals • 3 years old $\delta^{13}C$ was correlated with Se concentrations in muscle tissue from females. For narwhals > 3 years old $\delta^{13}C$ were correlated Hg and Se in muscle and kidney tissues of males.

***Table 3.4.3**. Correlation matrix of Pearson's correlation coefficient. * denotes 0.01<p<0.05, ** denotes p<0.01*

	Cd-M	Cd-L	Cd-K	Zn-M	Zn-L	Zn-K	Hg-M	Hg-L	Hg-K	Se-M	Se-L	Se-K
Female • 3 years old:												
$\delta^{15}N$	-0.55**	-0.79**	-0.88**	0.16	0.24	-0.71**	-0.29	-0.58*	-0.76**	-0.13	-0.70**	-0.76**
$\delta^{13}C$	0.26	-0.33	-0.33	0.33	0.27	-0.11	-0.06	-0.08	-0.16	-0.54*	-0.34	-0.15
Male • 3 years old:												
$\delta^{15}N$	-0.42	-0.80**	-0.84**	0.11	0.44	-0.52	-0.70**	-0.56*	-0.47*	-0.57*	-0.69**	-0.80**
$\delta^{13}C$	-0.28	-0.22	-0.19	0.16	0.27	-0.19	-0.08	0.00	-0.05	-0.10	-0.08	0.01
Female > 3 years old:												
$\delta^{15}N$	0.43**	0.20	-0.05	0.22	0.00	-0.08	0.21	0.17	0.02	0.43**	0.13	-0.17
$\delta^{13}C$	0.11	0.07	0.10	0.12	0.24	0.11	-0.08	0.03	0.19	-0.29	-0.17	0.29
Male > 3 years old:												
$\delta^{15}N$	-0.09	0.14	-0.13	0.12	-0.17	-0.14	0.36**	0.19	0.38**	0.33**	0.24	0.35**
$\delta^{13}C$	-0.08	0.16	-0.21	0.05	-0.11	-0.19	0.45**	0.19	-0.45**	-0.30**	-0.08	0.30**

POPs

Table 3.4.4 shows the correlation coefficients between log-transformed PCBs and stable isotopes. In females, $\delta^{15}N$ was generally positively correlated with POP groups although not significantly, while in males the correlation were negative and in some cases significantly. No explanation of this difference between females and males can be given. $\delta^{13}C$ was found to be only weakly correlated with POP groups with the exception of HCHs in males.

Summary

For $\delta^{15}N$ significantly higher levels were found in Uummannaq than in Avanersuaq indicating that Uummannaq narwhales feed on higher trophic levels, but the low degree of correlation between $\delta^{15}N$ and metal or POP levels is inconsistant.

*Table 3.4.4. Correlation matrix of Pearson's correlation coefficient. * denotes 0.01<p<0.05, ** denotes p<0.01.*

	PCBs	HCB	HCHs	DDTs	trans-nonachlor	Toxa-phene	CHBs
Females:							
$\delta^{15}N$	0.25	0.30	0.20	0.25	0.33	0.30	0.30
$\delta^{13}C$	-0.14	-0.09	-0.07	-0.14	-0.19	-0.06	-0.10
Males:							
$\delta^{15}N$	-0.45**	-0.18	-0.06	-0.45**	-0.24	-0.27*	-0.19
$\delta^{13}C$	0.05	-0.14	-0.30*	0.10	0.08	0.04	-0.05

3.5 DNA analysis

Low levels of variation

A total of 100 samples were sequenced yielding three haplotypes not found in the previous set of 70 sequenced samples (Palsbøll *et al.* 1997) (see Table 3.5.1). The haplotype of five of the 100 samples had previously been determined by RFLP analysis. The present sequence data set thus added 100 new sequenced samples to the 70 presented in Palsbøll *et al.* (1997). The total RFLP data set thus increased from the 427 samples in Palsbøll *et al.* (1997) to 522 (427 + 100 - 5, see Table 3.5.2).

Indications of a bottleneck

Table 3.5.1 lists the estimates of nucleotide diversity in the various partitioning obtained from either the sequenced samples only, or the total RFLP data set of 522 samples. No significant differences in the level of nucleotide diversity estimated from either kind of data were detected. The observed values were between zero and 0019 as reported by Palsbøll *et al.* (1997). This level of sequence variation in the mitochondrial control region is low relative to what has been reported for other cetacean species and probably due to a "recent" bottle neck, as suggested by Palsbøll *et al.* (1997).

Star-like genealogy

The eight narwhal haplotypes are all closely related only separated by a few nucleotide substitutions, which resulted in a near star-like genealogy (Figure 3.5.1) as is characteristic of a population expansion model (Slatkin & Hudson 1991).

3.5.1 Population structure

Overall population structure

The homogeneity tests of the expanded data set (with the samples sequenced for this contract) did confirm the general population structure hypothesised by Palsbøll *et al.* (1997). The authors suggested a local West Greenland population that summers in the Melville Bay area and winters in the Disko Bay. The narwhals sampled in the northern Baffin Bay (eastern Canada and the Avanersuaq district) differed in frequency composition from the "West Greenland" popu-

lation and the East Greenland samples (which all were haplotype MM002).

All-year North Water population

Samples collected from a winter sassat in the southern Avanersuaq district (Savissivik 1994) did have a similar haplotype frequency composition as the Canadian and summer Avanersuaq samples, indicating that some of these narwhals probably winter in the North Water polynia.

Mixing of winter grounds

The Disko Bay appeared to constitute a common winter ground as the two partitionings of samples collected there (a 1994 sassat and the remainder) differed significantly. Also they had a haplotype composition that resembled either the West Greenland samples (Melville Bay and Upernavik) or the northern Baffin Bay sample (eastern Canada and the Avanersuaq district), respectively.

Table 3.5.1 Sample sizes and haplotypic composition of sampling localities

Sample area	Haplotype								
	MM001	MM002	MM003	MM004	MM005	MM006	MM007	MM008	Total
Disko Bay	8/3/5	8/7/0	-	-	-	-	-	-	31
Disko Bay 1994 Sassat	18/21/0	5/6/0	-	-	0/1/0	-	-	-	51
Eastern Canada	13/9/0	4/1/0	-	0/1/0	0/1/0	-	-	-	29
Eastern Greenland	-	17/10/0	-	-	-	-	-	-	27
Balgoni Island	6/3/0	3/8/0	-	-	-	-	-	-	20
Ava. district 1984 & '85	33/30/10	5/3/0	3/2/0	1/0/0	-	0/1/0	2/0/0	-	90
Ava. district 1990	1/0/0	1/0/0	-	-	-	-	-	-	2
Ava. district 1993 & '94	37/55/1	14/23/1	1/0/0	-	3/0/0	-	-	-	135
Upernavik	0/5/0	1/3/1	-	-	-	-	-	-	10
Uummannaq	30/84/0	4/8/0	-	-	-	-	-	1/0/0	127

*Table **3.5..2.** Polymorphic nucleotide positions for the eight haplotypes detected in the 522 analyzed narwhals.*

Haplotype	Nucleotide position					
	27	58	115	189	230	265
MM001	C	-	A	G	C	C
MM002	.	A	.	.	.	T
MM003	.	.	.	.	.	T
MM004	.	.	.	.	T	.
MM005	.	.	G	.	.	.
MM006	T	.	.	.	.	T
MM007	.	.	.	A	.	.
MM008	.	.	.	.	T	T

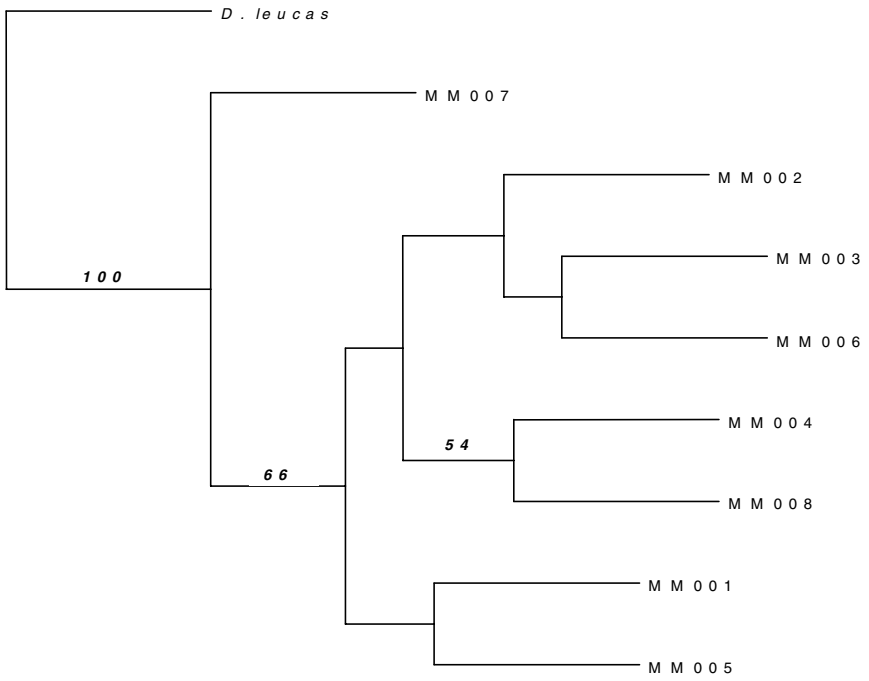

***Figure 3.5.1**. The genealogy estimated from the eight narwhal haplotypes and rooted with the homologous beluga sequence. Note: Numbers indicate bootstrap values in percent (only values over 50 included)*

Separate Uummannaq "population"

The samples collected in the Uummannaq district *d*uring the late autumn (November) differed significantly from all other sample partitioning and thus appeared to constitute a separate sub-population, which was consistent with the migration timing of individual narwhals radio tagged in the Melville Bay (Dietz & Heide-Jørgensen, 1995). The analyses by Palsbøll *et al.* (1997) did not detect any significant degree of inter-annual heterogeneity within one sampling area apart from the Disko Bay. However the outcome of such analyses relies greatly on the statistical power of the analyses, i.e. the sensitivity of the employed statistics, the degree of sequence variation and sample sizes.

Sequential Bonferroni correction

The homogeneity tests performed using the expanded data set yielded multiple comparisons with levels of genetic divergence (estimated as H_{ST}) that were associated with low P-values (<0.05, Table 4). The correct statistical procedure, when such simultaneous pairwise comparisons are conducted, is to perform sequential Bonferroni corrections (Rice 1989) to "weed out" random P-values, e. i., of every 100 tests we should expect five significant test if the chosen α-level is 0.05. However the degree of significance has to be taken into account as well, which is the measure employed by the sequential Bonferroni test. Of the 28 pairwise tests, 12 yielded P-values that were significant at a table-wide P-level of 0.05 (i. e. after Bonferroni corrections).

High level of significant population structure

Of the remaining 16 tests one or two P-values below 0.05 could simply be due to stochastic error. However, six of the 16 remaining tests have estimated P-values less than 0.05 (Table 3.5.3) indicating that some of these estimates are unlikely to be simple stochastic errors, but probably a product of low statistical power due to small sample

sizes. That this may be the reason becomes more apparent when considering the actual estimates of genetic divergence (the H_{ST} values as opposed to the associated P-values).

Degree of divergence

In general (Table 3.5.3) marginal P-values are associated with a substantial (in the current context) degree of genetic divergence (e. i., H_{ST} > 0.02) and a low sample size for one of the two partitionings. Thus it appears P-values below 0.05 probably reflect a significant degree of genetic divergence, which should become statistical more rigorous with larger sample sizes.

Within locality structure in the Avanersuaq district

The homogeneity tests thus indicated a high level of structure and even within sampling areas (Table 3.4.3). The new data set added a significant number of samples to the Avanersuaq district samples, especially from 1984 and 1985. A comparison of the combined samples from 1984 & 1985 with the samples collected in 1993 & 1994 in same area did yield a significant level of divergence (H_{ST}=0.021, P=0.009). This level of divergence was only slightly lower than that between e.g., Avanersuaq (1993 & 1994) and Uummannaq district (Table 3.4.3).

Population genealogy

The estimated genealogy of populations indicates three major partitionings; East Greenland, West Greenland and Baffin Bay. Especially among the Baffin Bay samples we detected a high level of fine-scale structure. However, the small number of samples from each locality within the West Greenland population does not enable a similar detailed analysis within this area and thus we cannot out rule (with the present data) that such exists.

3.5.2 Summary

The added data, generated by this project, did yield additional insight regarding the overall population structure of narwhals. Indeed the new expanded analyses revealed a higher level of structure than previously reported by Palsbøll *et al.* (1997). Three major population divisions were proposed; local East and West Greenland populations, as well as a Baffin Bay population. The West Greenland population appears to migrate along the coast wintering in the Disko Bay area and summering in the Melville Bay area.

The Baffin Bay population

The Baffin Bay population was represented by samples from eastern Canada, Avanersuaq, and Uummannaq among which a high degree of significant population sub-structure was detected. The sample sizes from the East and West Greenland populations did not enable a similar detailed analysis among samples from e.g., West Greenland for which reason we cannot out rule a similar level of structure within these populations.

Matrilineal structure

This high level of matrilineal structure may not be all that surprising given that narwhal pods of females and immature animals appear to be maternally structured (Hay & Mansfield 1989).

Within area structure

Thus it appears that a more comprehensive understanding of the population structure of narwhals may relay on mapping of migration

patterns, e.g., by radio satellite tagging as well as genetic analyses partly from under-represented sampling sites, and partly by detailed analyses of the genetic structure of different groups within single areas.

Within-pod consanguinity

This latter analysis is necessary to evaluate the level of inter-area divergence. In addition it will be necessary to analyse multiple biparental inherited loci (sufficient to determine parent-offspring pairs) within pods in order to determine if indeed these are maternal units.

Table 3.5.3. Estimated H_{ST} (above diagonal) and the level of significance (below diagonal).

	East Greenland	Kitsissuarsuit 1994	Disko Bay	Uumman-naq	Balgoni Island	Avanersuaq 1984 & 1985	Avanersuaq 1993 & 1994	Eastern Canada
East Greenland		0.53	0.36	0.61	0.27	0.55	0.27	0.64
Kitsissuarsuit 1994	> 0.0001		0.040	0.019	0.094	-0.008	0.002	-0.011
Disko Bay	> 0.0001	0.038		0.12	-0.0071	0.077	0.0077	0.059
Uummannaq	> 0.0001	0.04	>0.0001		0.19	0.0021	0.055	0.0024
Balgoni Island Upernavik	> 0.0001	0.007	0.60	>0.0001		0.14	0.34	0.12
Avanersuaq 1984 & 1985	> 0.0001	0.38	0.0010	0.30	>0.0001		0.21	-0.0075
Avanersuaq 1993 & 1994	> 0.0001	0.32	0.19	>0.0001	0.020	0.009		00031
Eastern Canada	> 0.0001	0.80	0.051	0.22	0.009	0.84	0.28	

4 Conclusions

Metal analysis

Cd, Hg and Se showed increasing concentrations in the first three to four years of life, after where the concentrations showed no change with age. Zn concentrations showed no apparent change with increasing age. For Cd the concentrations were significantly higher in females than in males.

Cd, Hg and Se were highly inter-organ correlated, whereas this was not the case with Zn. In liver and kidney several high correlations were found between Hg and Se and between Cd and Zn.

The comparison of metal concentrations between location was restricted to narwhals above 3 years old and the locations Avanersuaq and Uummannaq from where most data existed. Significant differences were found, however, it seems not to be related to the locality but rather the specific sampling (combination of location and sampling year).

In general, the metal levels found in narwhals from Greenland were within the range observed in Arctic Canada.

Persistent organic pollutants

Toxaphene was one of the dominant POPs as generally found in small toothed Arctic whales. PCBs in the Greenland samples seem to be a little lower than or at the same level as the Canadian samples, whereas HCHs and DDTs seem to be higher.

The dependence of age on POP concentrations was very different for the two sex. All POP concentrations in females showed a decreasing trend from the yearlings to about 8 to 10 years old. In males, PCBs, DDTs and trans-nonachlor showed an increase in the first few years, where after no increase or a small gradual increase with age was found. HCHs, total toxaphene and CHBs showed a decrease in the first few years followed by a gradual increase with age.

All POPs was highly inter correlated as expected.

No or only few statistically significant differences in POP concentrations was observed between locations. This is caused by the relatively low number of samples available when both sex and age have to be taken into account. However, it seems that the POP levels in general were highest in narwhals sampled at Balgoni Island 1993 and at Kitsissuarsuit 1990 and lowest in narwhals from Avanersuaq 1993. An analysis of the PCB congener pattern isolates the samples from Avanersuaq 1993 and from Kitsissuarsuit 1994 from each other and from the other samples. The PCB pattern in Avanersuaq 1993 narwhals was characterised by relatively high concentrations of both the higher chlorinated congeners ($Cl \geq 6$) and the two low chlorinated congeners (CB-28 and CB-31). Narwhals from Kitsissuarsuit 1994 had relatively low concentrations of the higher chlorinated congeners.

Stable isotopes

Stable isotopes ratios showed decreasing trend in the first year when they live of mother's milk where after they were relative stable with increasing age.

$\delta^{15}N$ was found to be significantly higher in Uummannaq 1993 narwhals than in Avanersuaq 1984 and 85 narwhals. This indicates that the Uummannaq narwhals feed on a relative higher trophic level than Avanersuaq narwhals. However, this was not reflected in the metal and POP concentrations.

$\delta^{13}C$ was found to be significantly higher in males than in females and significantly higher in narwhals from Uummannaq 1993 than in narwhals from Avanersuaq 1985 but not from Avanersuaq 1984. $\delta^{13}C$ is

an indicator of sources of primary production. No explanation of the observed results can be given.

The stable isotopes ratios ($\delta^{15}N$ and $\delta^{13}C$) were found to be only weakly correlated with metal and POP concentrations.

Genetic data

Genetic data obtained in this study was compared with previous data set from other areas of Greenland and Canada. Three haplotypes were observed which had not been found before. In general, the genetic variation was low relative to what has been reported for other cetacean species.

One local West Greenland population that summers in the Melville Bay area (sampled at Balgoni Island) and winters in the Disko Bay was identified. In addition a northern Baffin Bay population was identified. They may winter in the North Water and some also in the Disko Bay and mix with the local West Greenland population. Also a separate East Greenland population exists. However, some differences in genetic structure between years at a single sampling locality were observed.

Summary

The genetic investigation in this study gave the strongest indications of the occurrence of different populations of narwhals in the West Greenland area. The metal and POP levels could not support the existence of sub-populations. The reason may be that systematic differences in contaminants in the environment (prey items) in the distribution area of West Greenland narwhals are too small. Stable isotopes ratios indicate some differences in trophic position of the samples of narwhals, however, the metal and POP levels did not support this and these were, in general, not correlated with stable isotopes ratios.

5 Acknowledgements

DANCEA
Danish Cooperation for Environment in the Arctic
Ministry of Environment and Energy

The present project has been funded by the Danish Environmental Protection Agency as part of the environmental support program Dancea – Danish Cooperation for Environment in the Arctic. The authors are solely responsible for all results and conclusions presented in the report, and does not necessarily reflect the position of the Danish Environmental Protection Agency.

The financial support given by the Danish Environmental Protection Agency is gratefully acknowledged.

We extend our thanks to our laboratory technicians for their work on the metal and POP analysis and to Erik W. Born for supplying us with samples from the Avanersuaq area collected back in 1984.

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The hypothesis that different populations of narwhals in the West Greenland area exist has been tested by different biomarkers (metal and organochlorine concentrations, stable isotopes and DNA). Samples of muscle, liver, kidney, blubber and skin tissues of narwhals from West Greenland have been analysed for heavy metals, organochlorines, stable isotopes and DNA. The obtained results of metal concentrations and DNA were included in the existing database, whereas no previous data on organochlorines and stable isotopes in West Greenland narwhals existed.
The metal and POP concentrations and stable isotopes could not support the population structure with two West Greenland populations suggested by the genetic study.

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ISBN 87-7772-678-2
ISSN (electronic) 1600-0048