



## **Lipids and stable isotopes in marine food webs in West Greenland**

### **Trophic Relations and health implications**

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**National Environmental Research Institute**  
Ministry of the Environment · Denmark

# Lipids and stable isotopes in marine food webs in West Greenland

Trophic relations and health implications

*PhD thesis*

*Per Møller*



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**National Environmental Research Institute**  
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# Lipids and stable isotopes in marine food webs in West Greenland

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*PhD thesis*  
**2006**

*Per Møller*

**DTU**



PhD project carried out in collaboration between:

National Environmental Research Institute

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# Data sheet

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Abstract:	The potential use of lipids and stable isotopes as a source of information related to the West Greenland marine ecosystem (62°N to 72°N) including man, was investigated. Analysis were performed on marine tissues representing invertebrates, fish, seabirds and marine mammals as well as traditional meals from a local community. One part of the study also included minke whale samples from other part of the North Atlantic. Our results suggest a great potential in lipids and stable isotopes as a source of information in research issues related to a sustainable exploitation and management of the West Greenland marine ecosystem and to public health issues in Greenland. The results fill out an existing gap in our knowledge about the marine food web structure and trophic relations and add a potential new tool to improved management of large whales. In addition, data will be important when giving dietary recommendations, balancing the risk from the contaminants and the health-promoting fatty acids in the traditional diet of Greenlanders.
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**National Environmental Research Institute**

# 1 Preface

The present thesis entitled “Lipids and stable isotopes in marine food webs in West Greenland – trophic relations and health implications” is part of the requirements for obtaining a PhD degree at the Technical University of Denmark (DTU). The PhD programme was funded by the National Environmental Research Institute (NERI) and Greenland Institute of Natural Resources (GINR), and has been based on a number of research projects related to Greenland and the Arctic. The project has been supported 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 do not necessarily reflect the position of the Danish Environmental Protection Agency.

The study was performed at BioCentrum-DTU and The Centre for Advanced Food Studies, under the supervision of associate professor Lars Hellgren (main supervisor) and at NERI, Department of Arctic Environment, under the co-supervision of senior research scientists Rune Dietz and Poul Johansen. Additional co-supervision was received from senior research scientist Erik W. Born, GINR, Department of seabirds and mammals.

Field collections were planned and performed in close collaboration with GINR. Other institutes and scientists who contributed significantly to the study are:

Senior research scientist Kai Wieland, Departments of fish and shrimp, GINR (Food web study: Paper 1 and field collections). Senior research scientist Keith Hobson, Canadian Wildlife Services, Environment Canada (Food web study: Paper 1 and stable isotope analysis). Associate professor Bente Deutch and associate professor Jens C. Hansen, Center for Arctic Environmental Medicine, Aarhus University (Dietary studies: Papers 5 & 6). Research scientist Christian Sonne, Department of Arctic Environment, NERI (Immune response study: Paper 7).

Lyngby, 31 Maj 2006



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M.Sc. Per Møller

## 2 Acknowledgements

Through my work as a research assistant at NERI the opportunity for me to start on a PhD programme was realised through the support of my research director Jesper Madsen and director Klaus Nygaard at GINR. I am grateful for having been trusted this assignment and for their support through out the entire study.

I am most grateful to my supervisor Lars Hellgren for his support, inspiration and guidance. I know keeping me on track has not been an easy task. I appreciate the laboratory facilities that I have been trusted and the skilled assistance and supervision by the technical staff at BNG. It has been a tremendously learning experience. In particular I would like to thank Jesper Gøttsche, Grete Peidersen, Karen Jensen and Jannie Agersten for standing up with me, my many questions and samples. A special thanks to Lis Christensen for many and rich discussions, for teaching me all I know about gaschromatography, and for the assistance on method development and validation. In addition I would like to thank William W. Christie (SCRI) for comments and advice on lipid methodology.

Even though most of my time during this study was spent in the laboratories at BNG a great deal of inspiration and support has come from my co-supervisors Rune Dietz, Poul Johansen and Erik W. Born. Even though lipids has not been a key interest to them, their enthusiasm and early interest in this study has meant a lot to me, including introducing me to research in the Arctic in the first place.

This study has been based on collaboration with and contributions from a number of research projects and I would there fore like to thank all project leaders for taking me “aboard” and trusting me with their unique material. In this respect I would in particular like to thank Kai Wieland, Rune Dietz, Christian Sonne, Erik W. Born, Bente Deutsch, Jenc C. Hansen and Poul Johansen. These people and many more have contributed to the production of the scientific papers included in this thesis – I thank you all.

As an essential part of the data generated in this study I acknowledge the work performed by Keith Hobson on stable isotope analysis. A data-base was established in order to handle the large number of complex fatty acid data. Peter Mikkelsen (NERI, AM) has been responsible for the development of the database and his effort and contribution is much appreciated.

During fieldwork in Central and West Greenland I have recieved invaluable assistance from a number of local hunters and fishermen in and around Qaanaq, Uummannaq, Qeqertarsuaq, Saqqaq and Nuuk. In particular I would like to thank Johannes Lybert in Qeqertarsuaq for his great enthusiasm and skilled efforts.

Going on off shore field trips with RV “Paamiut” in the summer of 2003, a lot of extra hours and good work was invested by the crew and colleagues from GINR. I would like to thank every body in-



volved for a fantastic experience and a good job done. In particular I would like to thank cruise leaders Ole Andreasen (Danish Institute for Fisheries Research), Torben Henningsen (Zoological Museum, Copenhagen), Rikke Frandsen (GINR), Kai Wieland (GINR), Rasmus Nygaard (GINR) and Per Kannevorff (GINR) as well as the master of RV "Paamiut" and his crew for their patience and flexibility.

I would also like to thank scientific researcher Aqqaluq Rosing-Asvid and biology assistant Henning Matthæussen for their assistance on collections of hooded seal and walrus, and to senior scientific researcher Anders Mosbech (NERI) for the coordination of eider collections.

Assistance on logistic matters was often called for and in this respect I would like to express my appreciation and thanks to biology assistant Lars Heilmann.

Thank you to the many more who in some way or another have contributed to this study but are not mentioned by name including the staff at BNG, DMU and GINR.

During field activities in the Nuuk region I was given the opportunity to accommodate and work at GINR. My wife and children whom I brought along for a 4 week stay still speaks about people we met, got to know, and who learned us something new about Greenland and its people.

Last but not least I would like to thank my family. This study would not have been possible had it not been for the patience and understanding of my wife Shireen and my children Freja and Liv, at times when I was not there for them.

### 3 Summary

Lipids are essential to all forms of life. They maintain the structural integrity of cells, serve as highly concentrated energy storages and participate in many biological processes ranging from transcription of the genetic code to regulation of vital metabolic pathways and physiological responses. Hence, lipids represent an integrated response and adaptation of an individual to its surroundings and therefore bring a signal of roles not only at the individual but also at the community level.

Lipids are the source of energy storage and transfer in all Arctic food webs where limiting factors such as sunlight, temperature and ice are responsible for creating an unstable environment. Within the marine environment an adaptation has been in lipid composition, productivity and assimilation efficiency in primary producers and consumers with a positive effect on biodiversity and productivity in the areas. Apart from the productivity of these seas, humans have gained from the favourable lipid composition, which have been shown to have a potential beneficial effect on public health. However, some marine species in the Arctic used for consumption also contain rather high levels of contaminants with a potential negative effect on health.

To investigate the potential use of lipids and stable isotopes as a source of information related to the West Greenland marine ecosystem (62°N to 72°N) including man, we initiated a sampling of marine tissues from 42 marine species representing invertebrates, fish, seabirds and marine mammals as well as traditional meals from a local community. One part of the study also included minke whale samples from other part of the North Atlantic. The present thesis presents the first results of his material, and evaluates the use of lipids and stable isotopes in gaining information on food web and population structure, nutritional lipid quality and the effect of dietary changes.

Based on isotopic data we have established a food web model for the West Greenland marine ecosystem suggesting 5 trophic levels and so consistent with findings for similar high-latitude systems. However we identified the West Greenland food web to differentiate by a number of animals foraging at relative low trophic levels, hence suggesting a more efficient energy-flux through the food web. We have shown the potential of blubber fatty acid biomarkers in identification of stock structure and sub-populations of a large marine mammal, exemplified by the North Atlantic minke whale (*Balaenoptera acutorostrata*).

Having described the nutritional lipid quality of marine species of particular importance in the traditional Greenlandic diet, we have for the first time identified several food items with relative high concentrations of the highly bioactive fatty acids pristanic and phytanic acid. A method is suggested where lipid intake and quality is optimized taking contaminant levels in the diet into account. These data will be important when giving dietary recommendations, balancing the risk from the contaminants and the health-promoting fatty acids in the

traditional diet. We have analysed nutrients and contaminants in traditional meals and compared these to similar data 30 years ago and found that local food intake has decreased and with it the content of n-3 polyunsaturated fatty acids. Concentrations of contaminants in local food items have not decreased, except for PCB and lead.

The effect of a contaminated diet on the immune response in a predatory mammal has been investigated. A highly contaminated marine diet caused an impairment of both the nonspecific and specific cellular immune system in the West Greenland sledge dog (*Canis familiaris*). The study suggests that the high content of long-chained polyunsaturated fatty acid may be of importance when investigating combined immunotoxic effects of contaminated marine food.

In conclusion, our results suggest a great potential in lipids and stable isotopes as a source of information in research issues related to a sustainable exploitation and management of the West Greenland marine ecosystem and to public health issues in Greenland. The results fill out an existing gap in our knowledge about the marine food web structure and trophic relations and add a potential new tool to improved management of large whales. In addition, data will be important when giving dietary recommendations, balancing the risk from the contaminants and the health-promoting fatty acids in the traditional diet of Greenland.

# 1 Dansk resumé

Lipider er essentielle for alle livsformer. De opretholder cellers strukturelle integritet, tjener som et koncentreret energilager og deltag i mange biologiske processer lige fra transkription af den genetiske kode til regulering af vitale metaboliske processer og fysiologiske responser. Dermed repræsenterer lipider en integreret respons og tilpasning af en organisme til dennes omgivelser.

Udover sin rolle som energikilde så repræsenterer lipider ligeledes energitransporten i alle arktiske fødekæder, og er derfor kendetegnet for disse ustabile systemer, hvor faktorer så som sollys, temperatur og is er begrænsende. Inden for det marine miljø har en særlig tilpasning været i lipidsammensætningen, produktiviteten og assimilationseffektiviteten i primærproducenter og -konsumenter med en følgelig positiv effekt på biodiversiteten og produktiviteten. Ud over produktiviteten i disse farvande har befolkninger i Arktis haft gavn af den favorable lipidsammensætning der er påvist at have en potentiel gavnlig effekt på folkesundheden.

Med henblik på anvendelse af lipider og stabile isotoper som informationskilde relateret til det vestgrønlandske marine økosystem (62°N – 72°N) og dets befolkning, iværksatte vi en indsamling af væv fra 42 marine arter (invertebrater, fisk, havfugle og havpattedyr) samt måltidsprøver fra den lokale grønlandske befolkning. En del af studiet inkluderede ligeledes prøver fra vågehval taget i andre dele af Nordatlanten. Denne afhandling præsenterer de første resultater der bygger på dette materiale, og evaluerer i den forbindelse anvendelsen af lipider og stabile isotoper som informationskilder og ved tilegnelse af ny viden på områder vedrørende fødekæde- og populationsstrukturer, ernæringsrelateret lipidkvalitet og effekter af ændringer i kosten.

Vi har, på basis af stabil isotopdata, opbygget en fødekædemodel for det vestgrønlandske marine økosystem. Modellen antyder 5 trofiske niveauer og er dermed i overensstemmelse med tilsvarende arktiske systemer. Vi fandt dog ligeledes at den vestgrønlandske fødekæde kendetegnede sig ved et antal arter der fouragerede på et relativt lavt trofisk niveau, hvilket antyder en mere effektiv energiflux gennem denne fødekæde. Vi har ligeledes påvist fedtsyrer i hvalspæk, som potentielle biomarkører til identifikation af bestands- og populationsstrukturer for store marine pattedyr.

Efter at have beskrevet den ernæringsmæssige lipidkvalitet for marine arter, med særlig betydning i for den traditionelle grønlandske kost, har vi som de første identificeret adskillige fødeemner med relativt højt indhold af de særligt bioaktive fedtsyrer, pristan- og fytansyre. En metode er blevet præsenteret, hvor lipidindtaget og kvaliteten er optimeret og hvor der er taget højde for kontaminantniveauerne i kosten. Vi har analyseret for næringsstoffer og kontaminanter i traditionelle måltider og sammenlignet disse med tilsvarende data indsamlet for 30 år siden og fundet, at indtaget af lokal føde er faldet og sammen med den indtaget af n-3 flerumættede fedtsyrer (PUFA).

Kontaminantkoncentrationerne er, bortset fra PCB og bly, ikke faldet i den lokale kost.

Effekten af en kontamineret kost på immunresponsen i et rovpattedyr er blevet undersøgt. En højt kontamineret marin kost forårsagede en hæmning af såvel det specifikke som det non-specifikke immunrespons i vestgrønlandske slædehunde (*Canis familiaris*). Studiet antyder at det høje indhold af langkædede n-3 PUFA kan være af betydning når der kigges på den kombinerede immuno-toksiske effekt af kontamineret marin kost.

Vores studie har således påvist det store potentiale der ligger i anvendelsen af lipider og stabile isotoper inden for udforskning af bæredygtig udnyttelse og forvaltning af det vestgrønlandske marine økosystem og i forhold til folkesundheden i Grønland. Resultaterne udfylder et eksisterende tomrum i vores viden omkring den marine fødekædestruktur og trofiske relationer, og tilføjer et potentielt nyt værktøj til forbedring af forvaltningen af store hvaler. Ydermere vil data være vigtige når der fremover gives kostanbefalinger, hvor der skal balanceres mellem risikoen fra kontaminanter og de sundhedsmæssige fordele ved fedtsyrerne i den traditionelle grønlandske kost.

## 5 Structure of the thesis

This thesis consists of a general introductory chapter and 7 scientific papers. The introductory chapter is basically divided into three parts.

The first introductory part gives a brief overview of the circumstances and scientific context that motivated the research on which this thesis has been based.

The second part summarizes and discusses the main results of the scientific papers produced in this study. The most important findings are outlined together with conclusions and significance of the work. Sections have been arranged in such a manner that one serves as background and introduction to the following. This arrangement also determines the order of the seven manuscripts though it does not reflect the order in which they were written.

Finally, the third part evaluates and concludes on the overall results and outlines some perspectives for future research.

In the introductory chapter the findings of the papers will be cited by referring to the specific number of the paper in question as shown below.

### List of scientific papers included:

- |         |  |
|---------|--|
| Paper 1 | Møller P., Wieland K., Born EW., Hobson K., Nielsen TG., Mosbech A. and Hellgren L., (in prep.). An isotopic food web model for the West Greenland marine ecosystem.   |
| Paper 2 | Møller P., Born EW., Dietz R., Haug T., Ruzzante DE. and Øien N. (2003). Regional differences in fatty acid composition in common minke whales ( <i>Balaenoptera acutorostrata</i> ) from the North Atlantic. J. Cetacean Res. Manage. 5(2):115–124.                       |
| Paper 3 | Born EW., Riget FF., Kingsley MCS., R. Dietz R., Haug T., Møller P., Muir DCG., Outridge P. and Øien N. (in press). A multi-elemental approach to identification of subpopulations of North Atlantic minke whales ( <i>Balaenoptera acutorostrata</i> ). Wildlife Biology. |
| Paper 4 | Møller P., Hellgren L. and Johansen P. (in prep.). Nutritional lipid quality of marine resources in West Greenland.  |
| Paper 5 | Deutch B., Dyerberg J., Pedersen HS., Møller P., Aschlund E. and Hansen JC. (submitted). Dietary composition and health indicators in North Greenland in the 1970's and today. Nutrition Research.   |

- Paper 6 Deutch B., Dyerberg J., Pedersen HS., Asmund G., Møller P. and Hansen JC. (in press). Dietary composition and contaminants in North Greenland in the 1970's and 2004. Science of The Total Environment.
- Paper 7 Sonne C., Larsen HJ., Loft KE., Kirkegaard M., Letcher R., Shahmiri S. and Møller P. (2006). Impairment of cellular immunity in West Greenland sledge dogs (*Canis familiaris*) dietary exposed to polluted minke whale (*Balaenoptera acutorostrata*) blubber. Environ. Sci. Technol. 40:2056-2062

## 6 Focus and aims

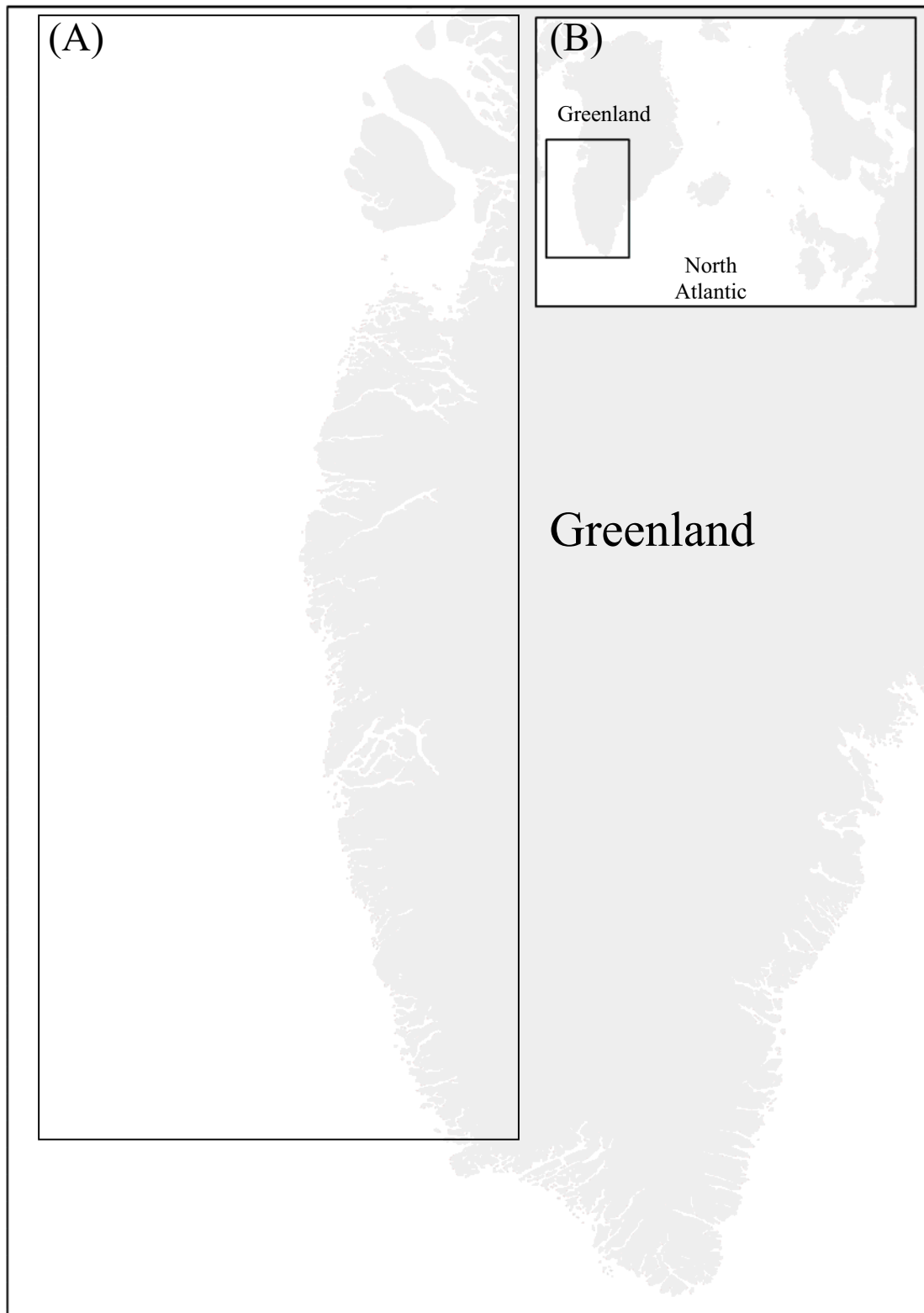
An over all aim of this study has been to explore stable isotope and fatty acids signatures as a source of information related to the West Greenland marine ecosystem including man. The primary focus and my main effort has been on the analysis of lipid content and fatty acid composition of tissues of marine animals, dietary meals and food items in West Greenland. Stable isotopes analyses are also presented. In particular:

1. Muscle and soft tissue from 44 marine species (invertebrates, fish, shark, seabirds and mammals) have been analysed for stable-nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) and stable-carbon ( $^{13}\text{C}/^{12}\text{C}$ ) in order to explore feeding habitats and the food web structure of the West Greenland marine ecosystem (Paper 1)
2. Superficial and deep blubber from 178 North Atlantic minke whales (*Balaenoptera acutorostrata*) have been analysed for fatty acid composition, in order to explore the potential use of fatty acid signatures in stock discrimination of large migratory whales (Paper 2 & 3).
3. Muscle, soft tissue and liver from 29 marine species (invertebrates, fish, seabirds and mammals) have been analysed for lipid content and fatty acid composition, in order to explore the nutritional lipid quality of marine resources available to the Greenland population (Paper 4).
4. Meals from 30 Greenlanders from Uummannaq town, West Greenland, have been analysed for lipid content and fatty acid composition, in order to explore the dietary development in Greenland over the past 30 years (Paper 5 & 6).
5. Minke whale blubber, pork fat and two types of dog pellets representing food components fed to West Greenland sledge dogs (*Canis familiaris*) have been analysed for lipid content and fatty acid composition, in order to administer energy intake and to explore differences in n-3 PUFA intake potentially affecting the immune response in an Arctic top predator (Paper 7).

With respect to focus and aims this thesis is divided into two parts. Part 1 is focusing on ecosystem issues, more specifically food web structure, foraging habitat and trophic relations (Paper 1) and fatty acid signatures as potential biomarkers (Paper 2 & 3). Part 2 is aiming at a description of lipid content, composition and quality in marine Greenland diet from a human health perspective (Papers 4-7).

The study area includes mainly the region of Southwest and central West Greenland i.e. Davis Strait (Paper 1-7) but in two of the papers (Paper 2 & 3) other North Atlantic regions are also included (Figure 1). The various methods used in this study will not be presented here but detailed descriptions are available from the scientific papers included in this thesis. However, before a presentation of our findings some important definitions and initial considerations on methodology are presented in brief.





**Figure 1.** Map of study area. The study area includes mainly the region of Southwest and central West Greenland (A) (Paper 1-7) but in two of the manuscripts (Paper 2 & 3) other North Atlantic regions are also included (B).

## 7 Definitions and initial considerations

### Defining lipids and fatty acids

Dictionaries, text books and most scientists choose a very general and loose definition, where lipids are described as naturally occurring compounds that are soluble in and can be extracted by organic solvents such as hydrocarbon, benzene, ethers, chloroform, alcohols etc. and as insoluble in water. However, many of the components that are now widely accepted as lipids are almost as easily dissolved in water (e.g. free fatty acids).

In this thesis I refer to an alternative definition set forward by W. W. Christie (Christie 2003), where lipids are defined as:

*"Fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds"*

This definition is based up on chemical structure and function and therefore seems more appropriate in a biological context since e.g. lipophilic contaminants and petroleum products are excluded by definition. It however does not include all natural substances like steroid hormones, some fat-soluble vitamins and carotenoids or terpenes.

Derived from the above definition, fatty acids are defined as:

*"Compounds synthesised in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex"*

### Initial considerations on methodology

Prior to the initiation of field collections and lipid analysis a number of considerations were made in order to ensure authenticity and quality of the analytical data. The most important ones are outlined below.

#### Sampling and storage

Due to the presence of double-bonds polyunsaturated fatty acids (PUFA) auto-oxidation will take place quite rapidly in air and it may not be possible to obtain an accurate analysis by chromatographic means. Auto-oxidation is exacerbated by strong light and metal ions and once initiated the process continues auto-catalytically (Christie 2003). Since marine organisms contain large amounts of unsaturated FA (up to 88%, Paper 4) and a large proportion is PUFA (up to 60%, Paper 4) caution has to be taken at all steps. Special caution was taken up to the time where lipids had been extracted and frozen. In most cases it was possible to collect the samples immediately after the

catch or preparation of the meal. Samples were then packed in polyethylene bags with air excluded and deep frozen (-28/-80°C) at the sampling site.

### **Sub-sampling**

Based on our understanding of the heterogeneous structure of biological material a sample must never be assumed to be homogenous. As part of the preparative work prior to lipid extraction a big effort was therefore made to get representative sub-samples. In brief whole semi-frozen samples were homogenised using a range of meat minzers, depending on the size and nature of the sample. Material was then withdrawn from 3-5 different parts of the homogenate to give sub-samples of up to 7g. Sub-samples from blubber were taken directly from the deep frozen material (-50°/-80°C) and represented complete cross-sections going from the deep layer adjacent to the muscle core to the superficial layer adjacent to the skin. In the study on minke whales of the North Atlantic (Paper 2 & 3) sub-sampling were targeted directly towards the two distinct layers. In all cases sub-samples were immediately taken to lipid extraction.

### **Lipid extraction**

A range of extraction procedures are available from the literature (Christie 1993a). Due to the use of less hazardous solvents the method by Smedes and Askland (1999) was considered but since it has proven to be less effective in the recovery of phospholipids (Smedes 1999) the method by Folch *et al.* (1957), most commonly used, was chosen. Methods do exist where lipids are simultaneously extracted and fatty acid methyl esters produced. However in my analytical setup analysis of lipid content and lipid class composition was conducted and total lipid extracts therefore had to be produced before an aliquot could be taken on to methylation. In the study on minke whales of the North Atlantic (Paper 2 & 3), this was not the case, and a one-step extraction and trans-esterification method (Sukihaja and Palmquist 1988) was chosen and modified for use on blubber.

The Folch extraction method was originally developed for the preparation of brain lipid (Folch *et al.* 1951) and has subsequently been simplified, the washing procedure optimised and its application on various animal tissues validated (Folch and Lees 1951, Folch *et al.* 1957). In brief, the method involves two operational steps. In the first step lipids are extracted from wet tissue by homogenisation in 20 fold 2:1 chloroform:methanol (C:M, v/v) followed by filtration of the lipid extract. During the second step the extract is cleaned from non-lipid substances by washing with a weak saline solution ending up with a final ratio of 8:4:3 (C:M:H<sub>2</sub>O, v/v/v). A two-phase system is formed with lipids in the lower phase. It is of outmost importance to keep the 8:4:3 ratio since a violation will result in the loss of lipids to the upper phase.

### **Fatty acid derivatives**

In order to analyse the fatty acid composition, fatty acids are often esterified to produce fatty acid methyl-esters (FAME). A number of

other derivatives are possible but based on the superior chromatographic properties of FAME these are most often the choice. Alternative derivatives like fatty acid butyl-esters (FABE) result in heavier fatty acid esters with changed physical properties (i.e. boiling point, polarity). This type of derivative is most often chosen when short-chained volatile fatty acids have to be considered. Structural clarification of fatty acids is sometimes necessary and for this purpose mass-spectrometry (MS) is a common tool. However FAME are not the best suited derivatives for this type of analysis, since double-bonds tends to migrate when exposed to electronic ionisation.

For the analysis of fatty acid composition I chose FAME and for structural clarification both picolinyl, pyrrolidid and DMOX derivatives were applied. Only initial considerations regarding the procedure for FAME is given below.

A two-step saponification and methylation procedure was used for the production of FAME. In the first step NaOH-methanol was added to the lipid extract and left at 90°C for 10 minutes to give free fatty acids. The method was modified from that of Christie (Christie 1993b) by the addition of toluene at a ratio of methanol: toluene 7:3 (v/v) and was done in order to ensure solubilisation of the non-polar cholesterol esters (CE). This modification had been suggested through personal communication with Dr. W.W. Christie (Scottish Crop Research Institute, Dundee, Scotland, UK) and was validated to yield a conversion of >90% for a maximum of 0.5mg CE (unpublished data). During the second step free fatty acids were converted to FAME with in 2 minutes (90°C) using BF<sub>3</sub>-methanol (Morrison and Smith 1964). Using this two-step procedure limited the time where PUFA were exposed to elevated temperatures and at the same time ensured an effective conversion of a complex matrix of lipid classes to FAME.

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## 8 Synopsis

### 8.1 Introduction

Several good reasons exist as to why one should go and study the role of lipids in the West Greenland marine ecosystem. Common to them all are (1) the importance of the West Greenland marine ecosystem to the population of Greenland and (2) the source of information which lipids represent. But most importantly is that (3) lipids are the source of energy storage and energy transfer in all Arctic food webs.

#### Lipid and their function

Lipids are essential to all forms of life and are found in micro-organisms, plants and animals as well as in degrading biological material. In living organisms they occur in all cell types and contribute as principle components of the membranes where they are essential in maintaining the structural integrity of cells. They serve as highly concentrated energy storages and participate in many biological processes ranging from transcription of the genetic code to regulation of vital metabolic pathways and physiological responses (Gurr *et al.* 2002). Other more specialised functions related to the marine environment are in adjusting buoyancy, long-term energy stores and as integumental waterproofing. In marine mammals lipids also function as insulation, high-energy transfer from mother to pup through lactation and in echolocation.

Hence, lipids represent an integrated response and adaptation of an individual to its surroundings and therefore bring a signal of roles not only at the individual but also at the community level.

#### Ecosystems and trophic relations

The Arctic is characterised by pronounced seasonal oscillations in light intensity and duration and combined with a dynamic sea ice regime which may vary considerably on an hourly to a decadal time scale (Murphy *et al.* 1995, Vinje 1999). This strongly influences the amount and quality of light available to primary producers in the marine environment (Falk-Petersen *et al.* 2000). As a consequence, pelagic animals in high-latitudes seas experience highly variable food supplies within, as well as between years (Lee and Hirota 1973, Falk-Petersen *et al.* 1990). Hence polar pelagic ecosystems are markedly unstable (Sakshaug *et al.* 1997) and pelagic algae and animals therefore need to be able to adapt to environmental changes on different time scales. As a consequence species in such unstable environments are forced to explore a relative wide ecological niche and this of course has fundamental implications for the biodiversity and bio-production of these ecosystems.

Despite of the relative short and intense periods of primary production, marginal ice zones are often among the most productive marine

systems of the Northern Hemisphere. In fact, low temperature and light intensity have been reported to favour lipid production in algae (Smith and Morris 1980). Under such conditions up to 80% of the carbon assimilated by algae is incorporated into the lipid fraction.

In marine environments, lipid content is related to modes of life (e.g. Friedrich and Hagen 1994, Auel and Hagen 2005), where enhanced lipid accumulation generally is associated with increased pelagic life-style. Pelagic species, especially at the lower trophic levels, have developed an ability to efficiently accumulate energy in the form of lipids whereas benthic invertebrates are generally poor in lipids. Hence marine phytoplankton and benthos have adapted differently to the extreme conditions of polar regions.

The characteristic of assimilating lipids is particularly effective in herbivorous zooplankton which also has a unique ability to store large amounts of lipids as energy reserves. This will allow them to survive periods of food shortage and to maintain a timely reproductive cycle. Furthermore, zooplankton is highly efficient in lipid production. This is exemplified by a lipid increase from 10-20% dw (50% PUFA) in primary producers (i.e. pelagic algae and ice algae) to >50% dw in pelagic herbivorous zooplankton and ice-fauna. This efficient production is rapidly transferred through the food chain to supply energy to higher trophic levels. This lipid-driven flux of energy is likely to be the key of biodiversity within polar systems.

In contrast to herbivorous copepods (e.g. *Calanus* and *Calanoides* species) calanoid copepods like *Paraeuchaeta* and *Euchaeta* contain rather high amounts of lipid throughout the year (Littlepage 1964, Lee 1974, Båmstedt and Matthews 1975, Båmstedt 1975, 1986, Hagen *et al.* 1995, Auel and Hagen 2005). The epipelagic species of these predators find optimal feeding conditions from early summer to fall, when herbivorous prey species, e.g. *Calanus* spp., respond to the short and intense phytoplankton bloom and start reproduction (Alonzo *et al.* 2000). In addition, the ascend of over wintering copepods in spring contributes to higher prey concentrations in the surface layers. Mesobathypelagic species however, face better feeding conditions during fall and winter, when dominating herbivorous copepods (e.g. *Calanus* spp. and/or *Calanoides*) descend to over winter at these depths. Hence, through these and other predators feeding on herbivorous zooplankton the herbivorous lipid energy stores generated by the short and intense phytoplankton bloom is canalised to more lipid constant resources available to higher animals in both the upper and lower water masses.

Lipids play an important role in the productivity of pelagic systems not only quantitatively but also qualitatively. Strong indications exist that lipid quality based on PUFA and especially the highly-unsaturated fatty acids (HUFA) i.e. 20:5n-3 and 22:6n-3 are essential and can limit zooplankton productivity (Jonasdottir *et al.* 1995, Müller-Navarra 1995). In fact low energy transfer between primary producers and consumers have been related to low relative 20:5n-3 content of the primary producer community (Müller-Navarra 2000). Thus, 20:5n-3 seems to be of general importance for the trophic transfer of energy and elements within aquatic food webs (Brett and Mül-

ler-Navarra 1997) both at low and high food concentrations. In this respect protozoa, known for their intermediate trophic role in transferring organic matter from small size planktonic particles to mesozooplankton, has been shown to biochemically upgrade inadequate food to high-quality copepod food (Klein Breteler *et al.* 1999). Through this mechanism, they have been assigned the function as trophic upgraders bridging the gap of essential nutrients between the microbial loop and higher trophic levels.

Marine food webs contrasts with terrestrial systems in that the primary producers are unicellular phytoplanktonic algae relative rich in lipids (10-20% dw) and poly-unsaturated fatty acids (PUFAs) 20:5n-3, 22:6n-3, C16-PUFA and C18-PUFA (50% of total fatty acid). Green terrestrial plants, including vegetables, have comparatively more PUFA (60-80% of total fatty acids) but this fraction is dominated by C18:3n-3 and due to the low lipid content (generally <2% dw) is of quantitatively little importance (Møller *et al.* 2005). Agricultural food production also produces vegetable seed oils rich in the mono-unsaturated fatty acids (MUFA) C18:1n-9 and the PUFA C18:2n-6. Some seeds like rapeseed and linseed also have considerable amounts of C18:3n-3 but are then accompanied by large amounts of 18:2n-6 (Møller *et al.* 2005).

In animal food production, the relative small amounts of 18:3n-3 fed to mainly ruminants are readily bio-hydrogenated to C18:0 by rumen microorganisms (Scollan *et al.* 2001) and results in a terrestrial agricultural food production that produces primarily animal fats rich in saturated fats, mainly C16:0 and C18:0.

In marine food chains bio-hydrogenation reactions do not occur. This characteristic combined with the effective lipid accumulation and lipid-flux up the food chain results in marine organisms being rich in PUFA and especially in 20:5n-3 and 22:6n-3.

The relative abundance of 20:5n-3 and 22:6n-3 in the marine primary production depends on the species composition of phytoplankton and may vary spatially, temporally and seasonally. The fatty acids 20:5n-3 and C16 PUFA are generally considered as diatom biomarkers whereas 22:6n-3 and 18:4n-3 are dino-flagellates biomarkers (Viso and Marty 1993).

The fact that each phylum of algae has a characteristic PUFA composition can be used to make direct correlation between the fatty acid composition of phytoplankton sampled in the field and the species present in the algae assemblage (Kattner *et al.* 1983, Pond *et al.* 1993).

It is generally recognised that specific physical processes determine the structure of pelagic plankton communities. One such community is based on diatoms (nutrient replenished “new” production) and is found in areas of mixed water regimes (frontal regimes). Another community is based on bacteria and flagellates (nutrient replete “re-generated” production) and is found in more stable water regimes (stratified regions) (Malone 1980, Legrendre 1981, Cushing 1989, Nielsen *et al.* 1993).



A basic adaptation in aquatic poikilotherms to a change in environmental temperatures is an increase in the proportion of PUFA in the polar lipids of biomembranes at low temperature (Cossins and Raynard 1988) and this phenomenon has been demonstrated in invertebrates, vertebrates and in photosynthetic algae (Henderson and Mackinlay 1989). An additional influence on lipid composition comes from changes in light and nutritional conditions as it has been shown in laboratory grown microalgae (Shifrin and Chisholm 1981, Thompson *et al.* 1990, Reitan *et al.* 1994).

Hence, changes in the lipid composition of phytoplankton can be related to changes in environmental parameters such as seawater temperature, sun light, sea ice and water masses (Kattner *et al.* 1983). Additionally a conservative transfer of fatty acids as food web indicators up to higher trophic levels and their application as biomarkers have been proven (e.g. Lee *et al.* 1971, Fraser *et al.* 1989, Graeve *et al.* 1994a, St. John and Lund 1996). Based on this phenomenon fatty acid biomarkers have been used to infer diet (Sargent *et al.* 1985, Kattner *et al.* 1989, Desvillettes *et al.* 1994, Nelson *et al.* 2001) and to deduce feeding strategies (Sargent and Falk-Petersen 1981, Graeve *et al.* 1994b) as well as a tool to discriminate between populations (e.g. K  kel   *et al.* 1993, Iverson *et al.* 1997, M  ller *et al.* 2003).

To be able to interpret the use of lipids and particularly fatty acids as biomarkers in marine ecosystems, information on the food web structure is important. In this respect naturally occurring stable isotopes of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$ ) have been used to trace primary productivity to and relative trophic level of organisms in marine food webs (Michener and Schell 1994). This approach is based on the principle that stable isotope ratios of consumer tissue can be related to that of diet (DeNiro and Epstein 1978, 1981). Between trophic levels an enrichment of 3-4‰ in  $^{15}\text{N}$  is generally observed (Michener and Schell 1994) and from this, relative trophic positions can be estimated for the establishment of food web models. Such stable-isotope based food-web models have given new information on contaminant, carbon and energy flow (Broman *et al.* 1992, Rolff *et al.* 1993, Atwell *et al.* 1998, Hobson *et al.* 2002, Buckman *et al.* 2004). Carbon shows little or no change in the relative abundance of  $^{13}\text{C}$  between primary producers and first level primary producers (Hobson and Welch 1992) and is therefore an indicator of sources of primary productivity in systems with isotopically distinct sources like phytoplankton vs. ice algae (Hobson *et al.* 1995). Additionally carbon isotope values are also enriched in inshore or benthic food webs when compared to pelagic food webs (Hobson and Welch 1992, Hobson *et al.* 1994, France 1995). Combining information on diet with stable-carbon and stable-nitrogen can provide valuable new information on trophic relations and feeding ecology (Hobson and Welch 1992, Hobson *et al.* 1994, Michner and Schell 1994, Kelly 2000, Lawson and Hobson 2000, Hobson *et al.* 2002).

### **Lipids and the traditional diet**

Lipids of marine origin have traditionally been the major energy source in the diets of the indigenous people in the Arctic areas of Greenland and North-America (Eidlitz 1969, Kemp 1984, Marquardt

1997). The fatty acid composition of these diets are characterized by low level of saturated fatty acids (SFA) and n-6 polyunsaturated (n-6 PUFAs), and high levels of monounsaturated (MUFA) and long-chained n-3 polyunsaturated fatty acids (n-3 PUFAs), compared with the western diets. However, the dietary habits of these peoples are at present in a rapid transition, going from the marine traditional diets, based in local culture and traditions, to a western diet, based on imported foodstuffs (Deutch 2002).

This dietary transition is mainly driven by the general socio-cultural changes towards a western life-style in the indigenous societies, but the focus on the potential health-risk linked to high concentrations of some contaminants in some marine diet items may further have decreased the consumption of these foodstuffs. In Greenland the traditional diet is still valued by people, but may result in a very high intake of contaminants (Johansen *et al.* 2004).

However, the traditional marine diet is very rich in the long-chained n-3 polyunsaturated fatty acids (n-3 PUFA), compared to the western diet (Bang *et al.* 1980), and as a consequence, the traditional food-pattern leads to a high plasma concentration of these fatty acids and a relatively low concentration of the long-chained fatty acid of the n-6 series (n-6 PUFA) (Dyerberg and Bang 1975). Therefore, the transition to a western-type diet leads to decreased level of plasma n-3 PUFAs, and an increased ratio between n-6/n-3 PUFAs, (Deutch *et al.* 2006a submitted), as well as an increased intake of saturated fatty acids (Receveur *et al.* 1997, Deutch *et al.* 2006a submitted).

Long-chained n-3 PUFA may reduce the risk of developing cardiovascular diseases (Yzebe and Lievre 2004). This has been verified just as an increased intake of n-3 PUFAs has proven to reduce the risk of sudden cardiac death and the risk of a fatal myocardial infarction (Yzebe and Lievre 2004). Furthermore, a daily n-3 PUFA supplements has been shown to reduce both systolic and diastolic blood pressure (Gelejsne *et al.* 2002).

Thus, the transition from a n-3 PUFA rich traditional diet to a typically n-3 PUFA-poor western diet is expected to have a negative impact on cardiac health. The negative health-effects of the dietary transition must be expected to be amplified through the general alteration in lifestyle, going from a hunter-gatherer subsistence with intense physical activity to a more sedentary western-type of salary-based economy.

### **Global change**

Changes in the abiotic factors due to climatic changes will affect the West Greenland marine ecosystem, its living resource and biological diversity. From 1920 a climatic amelioration markedly changed the marine fauna of West Greenland (Jensen 1939, Hansen 1949). The warming was reflected in the rise and fall of the fishery for Atlantic cod during 1950-1970 (Hansen 1949, Smidt 1983, Hamilton *et al.* 2003). This event was followed by a cooling of the northwestern Atlantic, including Baffin Bay and the eastern Canadian Sub-Arctic region from 1950-1990 (e.g. Grumet *et al.* 2001). Since 1990 temperature in the

Northern hemisphere, including Greenland, have increased markedly (Johannesen *et al.* 2004) and is expected to increase even further. Variations in the North Atlantic Oscillation (NAO) explain much of the variability in weather and climate in the North Atlantic (Hurrell 1995, Hamilton *et al.* 2003 and references therein). It fluctuates on a decadal time scale and may have been an important factor in climate change history of this area, however the variability has become particularly pronounced since 1950 (Hurrell 1995). Between ca 1980 and up till to day the sea ice area has decreased in the Baffin Bay/Labrador Sea (Comiso 2003), in parts of Baffin Bay and Hudson Bay (Liu and Curry 2004) and in Baffin Bay and Davis Strait (Johannesen *et al.* 2004). Hence, based on climatic changes and effects in the past, to days dramatic climatic changes observed in Arctic regions is currently affecting and will in the future affect the West Greenland marine ecosystem to an unknown degree.

### **Hypothesis and objectives of the present study**

This study was indented as a means of exploring stable isotopes and lipids as a source of information on issues related to a sustainable exploitation and management of the West Greenland marine ecosystem and to public health issues in Greenland.

Hence it was hypothesized that stable isotopes and fatty acids, and their signals, could be used as a tool for determination of:

1. the food web structure of the West Greenland marine ecosystem
2. stock discrimination in an important marine mammal component, the minke whale in the West Greenland marine ecosystem
3. nutritional lipid quality of marine species and contaminant-corrected best dietary marine sources to the Greenlandic population
4. changes in and current status of the Greenlandic diet
5. highly contaminated prey of marine origin and the effect on the immune response of an Arctic top predatory mammal.

## **8.2 The West Greenland marine ecosystem**

The climatic conditions of West Greenland marine ecosystem are highly variable both annually and on a decadal scale. In general, the conditions in West Greenland are influenced by an inflow of warm water of Atlantic origin. The Irminger current, a side branch of the North Atlantic current, brings warm and saline water of Atlantic origin up along the coast of West Greenland. From the Arctic Ocean surplus water is driven through the channel between Greenland and Canada and through the isles in the Canadian High Arctic into the Baffin Bay where it flows south along the east coast of Baffin Island and eventually becomes the Labrador Current. The strength of these currents determines the extent and development of sea ice conditions in southern Baffin Bay and Davis Strait. Sea ice conditions in Baffin Bay show some of the highest inter-annual variability in the Arctic (Mosbech *et al.* 2004a, 2004b). Between ca 1980 and to day the sea ice area has decreased in the Baffin Bay/Labrador Sea (Comiso 2003), in parts of Baffin Bay and Hudson Bay (Liu and Curry 2004) and in Baf-

fin Bay and Davis Strait (Johannesen *et al.* 2004). Hence, based on climatic changes and their effects in the past (Jensen 1939, Hansen 1949, Hamilton *et al.* 2003) today's climatic changes observed in Arctic regions (Johannesen *et al.* 2004, Comiso and Parkinson 2004, Hamilton *et al.* 2003) is currently affecting and will continuously affect the West Greenland marine ecosystem to an unknown degree.

Setting the exact geographical borders of "the West Greenland ecosystem" must necessarily be somewhat arbitrary due to the highly variable climatic conditions in West Greenland influencing temperature and ice conditions. Here I define the southern- and northernmost limits of the "West Greenland ecosystem" as 62°N and 72°N based on a combination of sea ice cover, currents and bathymetry (Hachery *et al.* 1954) (Paper 1). This area roughly represents 15% of the Greenlandic coast line and inhabits ca 75% (i.e. 38.000) of the population of Greenland.

The West Greenland marine ecosystem is highly productive and supports large populations of invertebrates, fish, seabirds and marine mammals. The banks along south western Greenland and the Disko Bay area are important spawning, nursery and fishing ground, especially for the northern shrimp (*Pandalus borealis*) and Greenlandic halibut (*Reinhardtius hippoglossoides*) fisheries that are central to the economy of Greenland (Buch *et al.* 2004, Simonsen *et al.* 2006). Through their early life the larvae are spread by the currents from the spawning grounds. Depending on their life stage they feed on plankton food or various benthic invertebrates. Knowledge about the trophic dynamics of the marine ecosystem from plankton to higher trophic levels is therefore essential for sustainable management of the exploitable living resources of the sea.

At present our knowledge on the Greenland marine food web, on which the important marine production is based, is relatively limited. In order to evaluate the biological effects of potential global changes it is important to have a basic understanding of the marine ecosystems in waters surrounding Greenland. Furthermore a sustainable exploitation of the marine resources has to be based on a basic scientific understanding of the ecosystem which makes the fundament of the production of these resources.

Previous efforts to gain information on trophic relations and the food web structure of the West Greenland marine ecosystem have mainly been based on traditional methods (i.e. stomach content, observations) and to my knowledge no previous attempts have been made to establish a marine food web model for this system.

In an isotopic food web study (Paper 1) we analysed stable carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and stable nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) in 42 species representing invertebrates, fish, seabirds and marine mammals.

Naturally occurring stable isotopes of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$ ) have been used to trace primary productivity to and relative trophic level of organisms in marine food webs (Michener and Schell 1994) and is based on the principle that stable isotope ratios of consumer tissue can be related to that of diet (DeNiro and Epstein 1978,

1981). Between trophic levels an enrichment of 3-4‰  $^{15}\text{N}$  is generally observed (Michener and Schell 1994) and from this, relative trophic positions can be estimated for the establishment of food web models. Additionally carbon isotope values are enriched in inshore or benthic food webs when compared to pelagic food webs (Hobson and Welch 1992, Hobson *et al.* 1994, France 1995). Combining information on diet with stable-carbon and stable-nitrogen can provide valuable new information on trophic relations and feeding ecology (Hobson and Welch 1992, Hobson *et al.* 1994, Michener and Schell 1994, Kelly 2000, Lawson and Hobson 2000, Hobson *et al.* 2002).

Stable isotope abundance was expressed in the  $\delta$  notation and used in the calculation of derived trophic levels (TL) according to Hobson and Welch (1992), Fisk *et al.* (2001) and Hobson *et al.* (2002). In the calculated TL we appointed the herbivorous *Calanus finmarchicus* to represent TL=2.0.

Based on the results we established an isotopic food web model for West Greenland that suggests a Sub Arctic marine ecosystem consisting of 5 trophic levels. This finding is consistent with the model established for the North Water marine food web (Hobson *et al.* 2002) and similar findings have been reported for the North East Water (Hobson *et al.* 1995) and the Lancaster Sound food web (Hobson and Welch 1992).

The fact that a Sub Arctic marine ecosystem like that of West Greenland is similar to three High Arctic systems in this respect, implies that abiotic factors influence these systems similarly, and they are therefore likely to respond to climatic changes in a similar fashion.

Noteworthy, our results also indicated that high-level species (i.e. *Uria lomvia*, *Phoca hispida*, *Delphinapterus leucas* and *Ursus maritimus*) seemed to be foraging at a lower trophic level when compared to e.g. the North Water food web. This first of all suggests a region specific diet but more importantly it also suggests a more efficient carbon and energy transfer in the marine food web of West Greenland.

Apart from gaining new information on the food web structure and trophic relations, the model was intended as a tool to assist in future work on modelling energy, contaminant flow and fatty acid biomarkers. In fact our isotopic model is already now being used in combination with fatty acid signatures to study and model mercury transport in the marine food web of West Greenland.

Species included in this model contribute to and explore the ecosystem differently depending on their abundance and foraging behaviour and e.g. migratory species like the minke whale (*Balaenoptera acutorostrata*) only occupy the West Greenland ecosystem part of the year. Many of the species are harvested either for local consumption or commercially for export purposes. This removal of biomass obviously also affects the ecosystem and the balance between ecosystem compartments.

Exploiting the marine resources of Greenland and doing so in a sustainable manner also implies management considerations based on the marine ecosystem as a whole and the populations within. How-

ever, in order to do so information like that gained from the food web study (Paper 1) is important because the distribution and abundance of the exploited species may fluctuate due to annual or long-term variations in abundance of lower trophic-level prey.

However, one of the management tools often used is based on information on population structure which is the subject of the next section where the use of FA for stock discrimination is explored.

### 8.3 Fatty acid signatures – a biomarker approach

In Greenland, the common minke whale (*Balaenoptera acutorostrata*) is exploited by subsistence hunters (IWC 2003, pp.68-70). Determining sustainable harvest levels however, requires an understanding of the population structure and the ability to identify the exploited units demographically. For management purposes, the International Whaling Commission (IWC) divided North Atlantic minke whales into four major management areas (“stocks”) based mainly on segregation by sex and length, catch distribution, marking data and the distribution of the whales at their summer feeding grounds and considerations of ecological conditions. These four “stocks” were: Canadian East Coast, West Greenland, Central Atlantic (East Greenland-Iceland-Jan Mayen) and Northeastern Atlantic (Svalbard-Norway-British Isles) (Donovan 1991a). These areas have been further divided into 10 “management sub-areas” of “small areas” (Anon. 1994, 2004)

Genetic data have proved equivocal information on stock structure (e.g. IWC 2004) and in order to improve the management of this species, it is therefore important that information from a variety of techniques is examined (e.g. Donovan 1991b). Other studies have applied various techniques including comparison of catch composition (e.g. Larsen and Øien 1988), morphological differences (Christensen *et al.* 1990) and reproductive parameters (Olsen 1997), but have not provided a definite answer to this question.

New analytical tools that reflect changes over a shorter time-scale compared to genetics may assist in the understanding of the population structure of North Atlantic minke whales. One such tool is the composition of fatty acids (FAs) in depot fats such as the blubber of marine mammals. Examples where FAs have been used as a tool to discriminate between populations include: ringed seals, *Phoca hispida* (Käkelä *et al.* 1993); harp seals, *Phoca groenlandica* (Grahl-Nielsen *et al.* 1993); harbour seals, *Phoca vitulina* (Smith *et al.* 1996, Iverson *et al.* 1997); and harbour porpoises, *Phocoena phocoena* (Møller *et al.* 2003). In addition, Olsen and Grahl-Nielsen (2002) were able to differentiate between minke whales from the Norwegian Sea and the North Sea using differences in FA signatures in blubber.

The method relies upon the fact that lipid composition of phytoplankton can be related to changes in environmental parameters (Kattner *et al.* 1983) and that a water mass related fatty acid signature is conservatively transferred as food web indicators up to higher trophic levels (e.g. Lee *et al.* 1971, Fraser *et al.* 1989, Graeve *et al.* 1994a,

St. John and Lund 1996). As a consequence the philopatric behaviour of minke whales is expected to reveal differences in use of habitats.

In a study on the population structure of minke whales in the North Atlantic (Papers 2 & 3) we investigated the potential of using blubber fatty acid signatures for the identification of sub-populations of marine mammals. For this purpose we analysed the fatty acid signature of deep and superficial blubber of 170 minke whales sampled in 1998 in West Greenland, the northeastern Atlantic and the North Sea. Fatty acid data was analysed in two ways.

In one analyses, fatty acid signature alone were used in combination with CART analysis (Paper 2). This analysis resulted in a suggested '3-Region Model' for the North Atlantic minke whale (*Balaenoptera acutorostrata*) stock i.e. (1) West Greenland, (2) a Central and Northeast Atlantic group (Jan Mayen, Svalbard, Barents Sea and north-western Norway) and (3) the North Sea. This is in accordance with IWC 'Medium Area' assumptions of three biological stocks (IWC 2004) not including Canada (no samples). Our findings using the fatty acid biomarker approach resembles those obtained in a genetic study using the same samples (Anderson *et al.* 2002).

The study indicated that fatty acid signatures of deep as well as superficial blubber can be used for identification of sub-populations of marine mammals.

In a second type of analysis a multi-element approach was investigated combining data on selected fatty acids from the signature of the superficial blubber with data on heavy metals (muscle, liver and kidney) and organochlorines (blubber) to reflect long-term deposition of 1+ year (Paper 3). The criteria that individual data should all be available from the same individual resulted in a reduction in sample size (n=104).

The assumption behind this study was that combining the signals from several elements and substances, including FA, would enhance the discriminatory power and thereby improve the ability to separate sub-populations.

Using a Canonical Discriminant Analysis (CDA) we were able to separate the whales into four sub-populations: (1) West Greenland, (2) a Central Atlantic group represented by whales from Jan Mayen, (3) a Northeast Atlantic group (Svalbard, Barents Sea and northwestern Norway), and (4) the North Sea. Basically this multi-elemental analysis supported the results of the genetic study (Andersen *et al.* 2003)

In an assignment test, 84% of the individuals were correctly assigned to the area they were sampled in. The highest degree of mis-assignment was found between Jan Mayen and the Northeast Atlantic group. The differences among the four groups likely reflected regional differences (i.e. sea water chemistry, prey type and prey availability) among the marine ecosystems within the range studied.

The study indicated that a multi-elemental approach including fatty acid biomarkers and based on supposedly long-term deposited com-

pounds with different ecological and physiological path-ways can be used for identification of sub-populations of marine mammals.

Based on the two approaches it was shown that more detail was achieved when fatty acid signatures were used in combination with other biomarkers. Since the signature from superficial blubber proved valid in both types of analysis we suggest that non-invasive distance sampling be considered in future studies.

## **8.4 Lipid quality of marine resources in Greenland**

We have demonstrated how fatty acids and their relative abundance in a marine tissue like blubber can be used as a source of information in marine mammal research. Another type of information to be gained from the fatty acid composition of a marine tissue is on the nutritional quality related to human public health. Consequently, the nutritional lipid quality of a marine resource can be determined and used as a tool in giving dietary advice. Assigning lipid quality also means having to distinguish between good and bad quality. Since the lipid quality of marine resources generally is considered very favourable to health, dietary advice should be based on a general comparison of dietary components.

The population of Greenland has recently gone through a rapid change in diet, moving away from a traditional marine diet to a more western-like diet, based on imported foodstuffs. This dietary transition is mainly driven by the general socio-cultural changes linked to a more western-like life-style, but awareness of contamination of the diet may also have had an effect. The traditional diet exposes Greenlanders to a high intake of heavy metals and persistent organic contaminants, but the traditional diet also has health-promoting properties. This “Arctic dilemma” motivated us to perform a study on the fatty acid composition and lipid content of marine key species of particular importance to the traditional diet of people in West Greenland.

In our study (Paper 4) we have investigated the lipid quality of muscle/soft tissue and blubber, since this is the quantitatively most important tissues in the diet. We analysed the lipid content and fatty acid composition of 29 marine species representing four taxa (invertebrates, fish, seabirds, marine mammals). Lipid quality was evaluated based on the content of essential fatty acids (EFA) and other potent fatty acids with documented health promoting effects, as well as the content of and balance between fatty acid classes. Lipid quality together with literature-based data on contaminant was evaluated to recommend marine resources for human consumption that both improve lipid quality and reduce contaminant exposure.

Our results showed that all species investigated had a high nutritional lipid quality, with potential positive implications to public health in West Greenland. The most favourable fatty acid composition, with low levels of SFA and high levels of MUFA and PUFA were observed in invertebrates, lean fish and blubber. As expected, the long-chained omega-3 FA dominated the PUFA fraction and



hence all dietary components represented a favorable balance between n-6 PUFA and n-3 PUFA with a ratio of 1 or less. When calculating the mass of n-3 PUFAs per kg wet weight, harp seal and ringed seal blubber were clearly the best sources. Furthermore, we identified several food items in the traditional diet with relative high concentrations of the highly bioactive fatty acids pristanic and phytanic acid, the richest sources being hooded seal and beluga blubber.

Based on our results we calculated contaminant-corrected LQCs corrected for Hg and PCB, respectively. By balancing lipid quality and contaminant exposure we have therefore been able to identify ringed seal blubber as the overall best source for PCB-corrected LQCs. Only for pristanic acid+phytanic acid was harp seal blubber picked as best source with ringed seal blubber as second best. For the Hg-corrected LQCs capelin was identified as the overall best source.

Based on our data on lipid quality components (LQCs) and data on contaminant content we suggest that contaminant corrected nutritional parameters are applied in future dietary models. The nutritional parameters should also be graded relative to their health risk/benefits in relation to public health. In addition, this model should include specific information on dietary recommendations related to population groups and diseases.

The fatty acid data generated in this study emphasize the high lipid quality of marine resources and their health implications as part of the traditional diet of West Greenland. Despite a pronounced difference in fatty acid signatures and balance between FA classes, all components represent a favourable balance between n-6 PUFA and n-3 PUFA (1 or less), and apart from blubber, Atlantic salmon, Arctic char and Greenland halibut, all are categorized as lean foodstuffs. Despite the high fat content in blubber, harp and ringed seal blubber is an excellent source of the n-3 PUFA, but blubber unfortunately also has high concentrations of persistent organic contaminants. The differences in the balance between FA and FA classes (SFA:MUFA:PUFA) should be considered advantageous as it allows for maneuverability when facing a number of different dietary scenarios. Hence, our data may be used to advice about diet in Arctic societies, aiming at the part of the population where diet and lifestyle imply a high risk of developing metabolic syndrome. Particularly under these conditions our results on the lipid quality of marine resources can be of assistance in giving targeted dietary advice, taking both the positive effects and the contaminant content into consideration.

## **8.5 Fatty acids and the Greenland diet**

The present dietary transition in the Greenlandic society, leads to decreased intake of marine foods. Hence, the dietary transition leads to a decrease in intake of contaminants, but also a concomitant decrease in n-3 fatty acids a situation commonly referred to as the "Arctic dilemma".

Scientific evidence has established that marine related lipids and especially the long-chained n-3 PUFAs EPA (20:5n-3) and 22:6n-3 (DHA) have a preventive effect on several of the pathologies related to the metabolic syndrome. Hence, they decrease plasma triglyceride (Hooper *et al.* 2004), decrease blood pressure (Geleijnse *et al.* 2002), endothelial-activation (Hjerkinn *et al.* 2005) and reduce the risk of cardio-vascular death (Albert *et al.* 1998, 2000, Hu *et al.* 2002). Implications of inflammatory responses have been related to the development of the metabolic syndrome (Sharma *et al.* 2005) and since n-3 PUFA have anti-inflammatory effects (Calder 2005) they might also in this case protect against the development of metabolic syndrome. The induction of weight loss and reduction in weight gain has been documented for EPA and DHA in rodents and humans, indicating an ability to protect against obesity-induced insulin resistance (Ruzickova *et al.* 2004, Couet *et al.* 1997).

Thus increasing the intake of EPA and DHA is likely to prevent development of cardio-vascular related pathologies in metabolic syndrome. Notably, it could also reduce the risk of developing obesity, thus preventing the development of the entire metabolic syndrome.

Lipid in the diet should represent no more than 30 energy percent and the balance between lipid classes SFA:MUFA:PUFA are generally recommended at 30:50:20. Also a diet with a ratio of n-6/n-3 (PUFA) between 1 and 4 is considered optimal (Simopoulos 2002 and references within). In this respect most marine species are considered lean and have an optimal n-6/n-3 balance (Paper 4).

Nutritional benefits and health implications related to the lipid quality of marine resources are potentially also valid for a diet including these resources. However, the lipid quality of other dietary components and the relative contribution to total dietary lipid content will determine the overall lipid quality exposure.

Information on the lipid quality of key marine resources in West Greenland has now been made available (Paper 4) and can be used in a more balanced dietary advice to the Greenland population. In order to give good dietary advice, though, it is important to have information on the dietary exposure of the person or population in question. This information should consist of both negative and positive elements in the diet.

In a dietary study we investigated the composition of traditional Greenlandic meals collected 30 years ago and to day's modern meals. The main objective was to gain information on the developmental trend and current nutritional status of the Greenland diet.

Dietary components, fatty acids, and nutrients in 177 traditional meals collected by duplicate portion method in Uummannaq municipality, north Greenland in 1976 were compared with 90 duplicate meals sampled in Uummannaq town in 2004. Anthropometric measures (weight, height, and body mass index, BMI) and blood lipids were measured as health indicators among the participants.

Our results reveal dramatic and significant differences in the dietary composition between the traditional food collected 30 years ago and

the food from 2004. The percentage of local food had decreased, and the content of n-3 fatty acids accordingly. Many vitamins and minerals had decreased, and were below Nordic Nutrient Recommendations in 2004. Vitamin A, B<sub>1</sub>, (B<sub>2</sub>), B<sub>12</sub>, iron, iodine, phosphorus, and selenium were correlated with n-3 content, whereas vitamin C, folate, and calcium were inversely correlated with n-3, but still low. The best balance between these two tendencies was found for medium intakes of n-3 (3-8 grams /day), corresponding to 20-40% local food. Body weight, body mass index (BMI), cholesterol, and S-triglycerides had increased significantly during the 30-years timespan.

In conclusion the dietary changes to a more western fare were found to be negative, resulting in less adequate nutrient coverage.

In the same study meals and blood samples from the participants were analysed for contaminants (i.e. 11 pesticides, 14 PCB congeners, heavy metals). Contaminant levels were adjusted for n-3 fatty acids, indicating local food content, and levels between 1976 and 2004 were compared.

Calculated as daily intake, all but three contaminants had decreased significantly. However, this could be explained by the lower intake of local food. After adjustment for n-3 fatty acid content in the food, significant declines of concentration in the local food were evident only for PCB and lead, whereas for mercury, DDTs, and chlordanes the levels were unchanged, and for hexachlorobenzene, mirex, and toxaphenes the levels had increased significantly.

From this it is evident that consumption of locally produced food has decreased in Greenland during the last 30 years and this has led to a reduction in the daily intake of contaminants. However, the concentrations of contaminants in local food items have not decreased, except for PCB and lead.

Based on this combined dietary study it has been recommended that the consumption of local products should not be increased beyond the present level, until the level of contaminants is reduced to a safer level (Paper 5+6). However, based on the newly emerged information on the lipid quality of the marine resources in Greenland and related health implications (Paper 4) I suggest that a revised recommendation should be made to encourage an increase above present levels. This revised recommendation should however be combined with a general revised dietary advice where highly contaminated tissues are replaced by less contaminated ones with a high LQ .

## **8.6 Marine foods and immune response**

The immune system is a complex interacting network of cells and bioactive factors which act to protect against bacteria, viruses, fungi, parasites, pathogens, micro-organisms, cancer cells and other similar attacks of danger to the health of the host. The cellular main components include granulocytes, mononuclear phagocytes and lymphocytes with further subdivisions according to functionality.

Generally speaking the function of the immune system is to identify "self" from "non-self" and to eliminate "non-self". It does so by recognising and responding to foreign antigens through the action of lymphocytes which form the core of the acquired and specific immune system. Upon an encounter of antigens lymphocytes communicate with other cells acting in the immune system. Communication within the acquired (specific) and between the acquired and natural (non-specific) immune system is brought about by cell to cell contact and chemical messengers. Cytokines represents one type of messenger and regulates the activity of cells producing cytokines and/or of other cells. Eicosanoids is another group of chemical messengers acting within the immune system. These are produced from PUFA and in particular arachidonic acid (AA) and eicosapentaenoic acid (EPA) and thereby provide a link between PUFA, inflammation and the immune system (Calder 2001). Fatty acid precursors for eicosanoid synthesis are released from phospholipids in cell membranes by the action of phospholipase A<sub>2</sub> activated in response to cellular stimulus (Calder 2001).

When these processes (i.e. recognising, communicating and responding) are working properly, the immune system decreases the incidence and severity of infections and is considered in balance. An imbalanced immune system over responds or does not respond enough.

Food components of either biological (i.e. nutrients) or anthropogenic (i.e. contaminants) origin affect the immune system in a number of ways. Nutrients most often help restore and maintain balance whereas contaminants are believed to result in an imbalanced immune system.

Since contaminants are found at relative high levels in marine foods, the immune system of Arctic top predators like the polar bear (*Ursus maritimus*) and humans inhabiting the Arctic is likely affected in a negative manner. It is generally accepted that the immune system is particularly sensitive to organohalogen contaminant (OHC) exposure (Tryphonas 1994, Vos and Luster 1989) and a number of studies on captive as well as free-ranging marine mammals suggest OHC related immuno-toxic effects, through mitogen-induced lymphocyte response and cell-mediated (lymphocyte proliferation) immunity (De Swart *et al.* 1994, 1995, Ross *et al.* 1995, 1996a, 1996b, 1996c). Similarly, a relationship between environmental organochlorine exposure and immuno-suppression has been suggested for humans as well (Dewailly *et al.* 2000, Morein *et al.* 2002).

Effects of fatty acids upon the immune system must also be considered, when dealing with food of marine origin. Especially the finding that n-3 PUFA found in fish oil can skew the profile of mediators (i.e. eicosanoids), that modulate inflammation and immunity (Lee *et al.* 1985, Sperling *et al.* 1993), has intensified the interest towards fatty acids and their effect on immune response. Since the discovery of the immuno-modulating properties of dietary fatty acids, a multitude of different immune functions has been shown to be affected by altering the balance between different fatty acid classes, such as the ratio between n-6 and n-3 PUFAs. More specifically, animal and human

studies indicate that decreasing the n-6 to n-3 PUFA ratio of the diet can decrease T lymphocyte proliferation, lymphocyte-derived cytokine production and the cell-mediated immune response (Calder 2001 and refs within). It is generally accepted that high levels of n-3 PUFA and a low n-6/n-3 ratio, as we find it in marine food (Paper 4-6), is beneficial and result in a balanced immune system. This is based on the observation of a decreased incidence of inflammatory and autoimmune diseases in populations like that of Greenland (Jørgensen *et al.* 2002). In fact the use of n-3 PUFA has been recommended to healthy populations to reduce the risk of autoimmunity and to prevent atherosclerosis (Ergas *et al.* 2002). However intake of a cocktail of components with an immune suppressive capacity, such as organohalogenes and n-3 fatty acids, a combined effect on the immune system may become adverse.

To determine if immuno-toxicity from OHCs in an Arctic top predator is a true cause-effect relationship, we conducted a study on domestic West Greenland sledge dogs (*Canis familiaris*) (Paper 7). Exposed groups were fed minke whale blubber, rich in mercury, OHCs, and n-3 fatty acids, while the control group was fed pork fat. The immune response after mitogen (PHA, Con A) and antigen (KLH) stimulation was measured using intradermal testing.

Our results showed that a daily intake of 50-200 g of minke whale blubber causes an impairment of the nonspecific and specific cellular immune system in the West Greenland sledge dog (*Canis familiaris*). Additionally, the relative high level of n-3 PUFA in minke whale blubber (17.5% vs 1.3% in pork fat), and the fact that n-3 PUFAs acts immunosuppressive and can lead to an impairment of host response (Fritsche *et al.* 1997, Paul *et al.* 1997) suggests that the immunomodulating effect of fatty acid is of importance and therefore should be considered when investigating combined immuno-toxic effects of contaminated food resources in future Inuit and polar bear studies. In fact the impairment of the immune response may have been caused through a combined effect of high levels of contaminants and n-3 PUFA in the food. However, the present data do not allow us to segregate the effects of the n-3 PUFAs and the contaminants.

We used contaminant exposure levels similar to those of Inuit and polar bears (*Ursus maritimus*), and it is therefore likely that Inuit and polar bears suffer from similar decreased resistance to diseases.

## 8.7 Conclusions and future research

In conclusion, the present work illustrates the importance of lipids and stable isotopes as sources of information related to marine food webs of West Greenland.

Remarkably little work has been done on the role of lipids in the West Greenland marine ecosystem despite their fundamental role in marine animals and the Arctic marine food web. Most of what is currently known represents work performed in the Canadian Arctic, Svalbard and Northern Norway and by a few research groups from countries with a long tradition within marine lipid research. Pres-

ently, however, research projects are emerging which include at least aspects of lipids in the West Greenland ecosystem. It is my hope that with this study awareness on the importance and potential use of lipids will add to this positive trend.

Combining stable isotopes and lipid biomarkers with other and more conventional tools will add new dimensions to the information level needed for a sustainable exploitation and management of the West Greenland marine ecosystem and its resources. At times where global change is at work and affecting the system to an unknown degree this approach is likely to give valuable new information on changes in food web structure, trophic relations, productivity and food quality of particular importance to the wealth and health of the Greenland population.

Evidence that the marine food web of West Greenland is similar in structure to that of Arctic food webs (Paper 1) may infer a number of similarities between these systems, however small deviations from that of high-Arctic polynyas are apparent, and may add significant new information to our basic understanding of these systems. Hence the fact that some higher level predators seem to feed at comparatively low trophic levels in the West Greenland system may prove essential. This is currently being investigated by the delineation of trophic relations through the combined use of our isotopic food web model and fatty acid biomarkers and is expected to give additional information on species-specific feeding strategies and diet. We suggest the marine isotopic food web model of West Greenland be used in future modelling of e.g. contaminant transfer, energy-flow and carbon-flux. Further development of the model aiming to include ideally all species (but especially invertebrates) is encouraged.

In order to exploit the marine resources of West Greenland in a sustainable way it is important to manage these on scientific based advice. On this basis, populations of large whales inhabiting Greenland waters have been managed for some time now where subsistence hunting has long been an important issue to the local populations inhabiting these areas. The International Whaling Commission (IWC) communicated the need for improved management tools in the management of the North Atlantic minke whale (*Balaenoptera acutorostrata*), currently being exploited by subsistence Norwegian and Greenlandic hunters. Conventional methods have failed to give significant new information and a search for new tools was encouraged. In this study we investigated the use of fatty acids and showed that blubber fatty acid signatures are a valid tool in stock discrimination in a large migratory whale like the North Atlantic minke whale (Paper 2). We recommend, however, that fatty acids be included in a multi-component approach (Paper 3) in order to ensure a more solid scientific basis for future advice. Apart from information on stock structure additional information can be gained from the use of these fatty acid signatures, due to the role and dietary origin of fatty acids. This combined potential deserves more attention in the future.

The role of marine species is essential in the traditional Greenlandic diet which is characterised by a high content of n-3 PUFA. Scientific evidence has established that marine related lipids and especially the

long-chained n-3 PUFAs EPA (20:5n-3) and DHA (22:6n-3) have a preventive effect on several of the pathologies related to the metabolic syndrome. However, compared to 30 years ago the Greenland diet of today has changed along with a more western-like lifestyle (Paper 5) and is no longer in agreement with international dietary recommendation. Fat intake is above recommendations and saturated fatty acids have increased at the expense of the nutritional desirable unsaturated fatty acids. This development has however resulted in a decrease in diet related contaminant exposure (Paper 6) but diseases related to the more western-like diet and sedentary lifestyle is likely to be the trade off. In fact an increase in type-2 diabetes and metabolic syndrome has already been observed in the Greenland population. This "Arctic dilemma" makes dietary advice quite difficult to give. Presently, a preliminary advice is that an increase in marine consumption should be avoided until contaminant levels have decreased to a safe level. Hence an increased frequency of life-style related diseases is expected among the Greenland population.

Both the fatty acid composition and the contaminant levels vary in marine species and in principle it should therefore be possible to compose a diet where the benefits and risks are better balanced. In order to do so marine species with a high lipid quality contrasting low contaminant levels needs to be identified.

From this study, information on the nutritional lipid quality of marine key species has been made available. Based on lipid quality components (LQCs) a number of best sources have been appointed after having been correcting for Hg and PCBs respectively. These contaminant-corrected LQCs can be used in giving a balanced dietary advice, where at the same time, contaminant exposure levels are reduced and the intake of healthy marine related lipids is increased, thereby circumventing the "Arctic dilemma". We suggest that contaminant-corrected LQC and other nutritional relevant parameters should be combined in a future dietary model for the Greenland population. Contaminants and nutritional parameters should be graded relative to their health risk/benefits in relation to public health. Finally a model should include specific information on dietary recommendations related to population groups and diseases.

Marine tissues and species not of quantitatively major importance to the current Greenland diet may prove as healthy alternatives and therefore serves more attention.

The health implications from a marine based diet is not one-sided and in this context lipids normally considered health may have negative effects on public health if total amounts are exaggerated. Apart from marine mammals blubber most marine food components are considered lean and would not challenge the recommended lipid energy percentage in the diet. Amounts of PUFA could however lead to an increased risk of peroxidation if antioxidant intake is not adequate. Evidence is increasing on the importance of balance between individual and classes of fatty acid in the diet. The anti-inflammatory effect of EPA can be advantageous to balance the immune system but if its counter-balance in the form of AA (20:4n-6) is low it may lead to impairment of the immune response. Contaminants and other nutri-

ents affect the immune response in a similar way and we have in fact shown that a marine based diet can impair the immune response of a predatory mammal. To what extent this was due to the high content of contaminants, EPA or low content of AA is not clear but most likely a combined effect of this cocktail is responsible for the effect. In order to investigate this further each factor has to be isolated and this will result in a daunting task but however more importantly contribute with a very significant peace of information.

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## 9 Scientific papers

- Paper 1     An isotopic food web model for the West Greenland marine ecosystem.
- Paper 2     Regional differences in fatty acid composition in common minke whales (*Balaenoptera acutorostrata*) from the North Atlantic.
- Paper 3     A multi-elemental approach to identification of sub-populations of North Atlantic minke whales (*Balaenoptera acutorostrata*).
- Paper 4     Nutritional lipid quality of West Greenland marine species.
- Paper 5     Dietary composition and health indicators in North Greenland, in the 1970's and today.
- Paper 6     Dietary composition and contaminants in North Greenland in the 1970's and 2004.
- Paper 7     Impairment of cellular immunity in West Greenland sledge dogs (*Canis familiaris*) dietary exposed to polluted minke whale (*Balaenoptera acutorostrata*) blubber.

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## **An isotopic food web model for the West Greenland marine ecosystem**

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Møller P., Wieland K., Born EW., Hobson K., Nielsen TG., Mosbech A.  
and Hellgren L.

In preparation.

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# An isotopic food web model for the West Greenland marine ecosystem

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## ABSTRACT

At present our knowledge of the Greenland marine food web is relatively limited. In order to evaluate the biological effects of potential global changes, it is important to have a basic understanding of the marine ecosystems in waters surrounding Greenland. Furthermore a sustainable exploitation of the marine resources has to be based on a basic scientific understanding. In order to gain new information on the food web structure and trophic relation of the West Greenland marine ecosystem, we aimed to establish a food web model based on stable isotope analysis.

Samples of muscle and soft tissue were collected off West Greenland, representing 42 marine species and 4 taxa (i.e. invertebrates, fish, sea-birds and marine mammals). Samples were analysed for stable carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) isotope using mass spectrometry. The  $\delta^{15}\text{N}$  was used in the assignment of individual trophic levels (TL) using the herbivorous copepod *Calanus finmarchicus* as reference (TL=2).

Mean  $\delta^{13}\text{C}$  values ranged from -20.5‰ to -15.8‰ with *Calanus finmarchicus* as the most depleted and snow crab (*Chionoecetes opilio*) as the most enriched species. Mean  $\delta^{15}\text{N}$  values ranged from 6.1-20.2‰ being most depleted in the suspension feeding blue mussel (*Mytilus edulis*) and most enriched in the top predator Polar bear (*Ursus maritimus*). Using  $\delta^{15}\text{N}$  values TL were calculated to range from 1.5-5.2.

Based on isotopic data we have established a food web model for the West Greenland marine ecosystem suggesting 5 trophic levels and so consistent with findings for similar high-latitude systems. However we identified the West Greenland food web to differentiate by a



number of animals foraging at relative low trophic levels, hence suggesting a more efficient energy-flux through the food web.

**KEY WORDS:** Food web model, Trophic level, Trophodynamics, Stable isotopes, Carbon-13, Nitrogen-15, Marine ecosystem, West Greenland.

## INTRODUCTION

The West Greenland marine ecosystem is highly productive and supports large populations of seabirds and marine mammals as well as several species of fish and shellfish of commercial importance.

The banks along south western Greenland and the Disko Bay area are important spawning, nursery and fishing ground, especially for the Northern shrimp (*Pandalus borealis*) and Greenland halibut (*Reinhardtius hippoglossoides*) fisheries that are central to the economy of Greenland (Buch et al. 2004, Simonsen et al 2006). Through their early life the larvae are spread by the currents from the spawning grounds. During their early life they are dependent of the plankton food until they settle to the bottom and feed on benthic invertebrates. Knowledge about the trophic pathways from the plankton through the higher trophic levels is therefore essential to manage and exploit.

Research activities in West Greenland and the Arctic in general have been increasing during the past decade mainly due to concern over the effects of global warming. Global warming will have the largest impact in Arctic regions but effects in the Arctic will potentially have global consequences (Hansen and Lebedeff 1987). Models have predicted, and observations already now confirm, a reduction in sea ice thickness and distribution (e.g. Johannessen et al. 1999, Kerr 1999) which will change the water balance and potentially have an effect on ocean currents globally (Schäfer et al. 2001). Locally a decrease in surface water salinity will result in a stronger stratification and a change in the longevity of the growth season of primary producers, thereby affecting the fundamental basis of the marine food web.

Changes in this marine ecosystem, where marine resources traditionally and financially are important, could also affect the culture, social structure and economic foundation radically. In Greenland a relatively large proportion of the local population live from subsistence hunting and fishing and the fishing industry in 2002 contributed 92% of the total export of Greenland (Anon. 2003).

At present our knowledge of the Greenland marine food web, on which the important marine production is based, is relatively limited. In order to evaluate the biological effects of potential global changes, it is important to have a basic understanding of the marine ecosystems in waters surrounding Greenland. Furthermore a sustainable exploitation of the marine resources has to be based on a basic scientific understanding of in particular the West Greenland ecosystem which is the head corner stone of the economy of present-day

Greenland. In this study we define the West Greenland marine ecosystem as the areas between ca. 62°N (Paamiut/Frederikshaab area) and ca. 72°N (Nuusuaq/Svartenhuk Peninsula) based on sea ice cover (i.e. minimal sea ice cover during winter), currents and bathymetry (coastal and continental shelf areas) (e.g. Hachery et al. 1954, Riget et al. 2000 and references therein). The vast majority of Greenlanders inhabit this area (Born 2000) which is greatly influenced by an inflow of relatively warm waters of Atlantic origin (Buch 2000) and therefore differs fundamentally from other areas in Greenland.

Naturally occurring stable isotopes of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$ ) have been used to trace primary productivity to and relative trophic level of organisms in marine food webs (Michener and Schell 1994). This approach is based on the principle that stable isotope ratios of consumer tissue can be related to that of diet (DeNiro and Epstein 1978, 1981).

Between trophic levels an enrichment of 3-4‰  $^{15}\text{N}$  is generally observed (Michener and Schell 1994) and from this, relative trophic positions can be estimated for the establishment of food web models. Such stable-isotope based food-web models have given new information on contaminants, carbon and energy flow (Broman et al 1992, Rolff et al 1993, Atwell et al 1998, Hobson et al. 2002, Buckman et al. 2004). Carbon shows little or no change in the relative abundance of  $^{13}\text{C}$  between primary producers and first level primary producers (Hobson and Welch 1992) and is therefore an indicator of sources of primary productivity in systems with isotopically distinct sources like phytoplankton vs. ice algae (Hobson et al 1995). Additionally carbon isotope values are also enriched in inshore or benthic food webs when compared to pelagic food webs (Hobson and Welch 1992, Hobson et al 1994, France 1995). Combining information on diet with stable-carbon and stable-nitrogen can provide valuable new information on trophic relations and feeding ecology (Hobson and Welch 1992, Hobson et al 1994, Michener and Schell 1994, Kelly 2000, Lawson and Hobson 2000, Hobson et al 2002).

The aim of this study was to establish a stable-isotope based food-web model in order to gain new information on the food web structure and trophic relations of the marine ecosystem of West Greenland. Additionally the model was developed as a tool to assist future work on modelling energy, contaminant flow and fatty acid biomarkers in this Sub-Arctic region.

## METHODS

### Field collections

In total, 42 species were included in our analysis. These are listed in Table 1 together with information on sampling area, size range, sampling period, sampling year, size range and sample size. Sampling areas (A-E) are given in Fig. 1. Invertebrate, fish and shark were sampled between 62° and 69°30'N (C-E), seabirds were sampled in area E

and marine mammal species represented the entire sampling range (A-E) up to 71°30'N (Fig. 1).

Twenty-nine of the 42 species (i.e. 6 of 9 invertebrates, fish, shark, 2 of 6 seabirds, 2 of 8 marine mammals) were collected during July-September. Deviations from this main sampling time window by the following species were unavoidable due to seasonal migrations. Copepods were sampled from late April to early June, while the remaining 4 species of seabirds and 6 species of marine mammals were sampled November-April (Table 1).

Samples of most of the marine fish species including Northern krill (*Meganyctiphanes norvegica*), Northern shrimp, snow crab (*Chionoecetes opilio*) and Boreoatlantic armhook squid (*Gonatus fabricii*) as well as minke whale (*Balaenoptera acutorostrata*) were collected in the offshore area while the remaining species were collected inshore. A more detailed description of field collections is given below.

Copepods were sampled from the research vessel R.V. Porsild (University of Copenhagen) in the Disko bay area off Qeqertarsuaq during late April – early June of 2005. Samples were taken at a permanent station located 1 nautical mile off Qeqertarsuaq (69°15'N, 53°33'W), which previously have been used for studying the pelagic community of Disko Bay (Nielsen and Hansen 1995, Levinsen et al. 2000, Madsen et al. 2001). The copepods were collected in the upper 50m of the water column using a WP-2 net (mesh size 200 µm). The samples were diluted in surface water in a 100 l thermo box and brought to the laboratory where the dominating *Calanus* species (*Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus*) were carefully sorted and rinsed in filtered surface water before transferred to a test tube and deep frozen (-28°C).

Samples of snow crab were collected in late summer 2003 during a routine pot survey using squid as bait (Carl and Burmeister 2005). Samples of Northern shrimp, Northern krill and Boreoatlantic armhook squid as well as muscle tissue of the majority of the marine fish species were collected in early summer 2003. Samples taken were deep frozen (-50°C) on board immediately after handling. A shrimp trawl with a relative high vertical opening (10-15 m) was used, which allowed to collect pelagic species such as Boreoatlantic armhook squid, juvenile redfish (*Sebastes* sp.), capelin (*Mallotus villosus*) and polar cod (*Boreogadus saida*) in addition to demersal/benthic fish (*Ammodytes* sp., *Sebastes mentella*, *Sebastes marinus*, *Reinhardtius hippoglossoides*, *Argentina silus*, *Hippoglossoides platessoides*, *Melanogrammus aeglefinus*, *Gadus morhua*, *Leptoclinus maculatus*, *Gadus ogac*, *Anarhichas lupus*, *Anarhichas minor*, *Myoxocephalus scorpius*) and shellfish (*Meganyctiphanes norvegica*, *Pandalus borealis*). Details on the fishing practice of this survey can be found in Kanneworff and Wieland (2003). Both, the snow crab survey and the bottom trawl survey for Northern shrimp and fish are routinely conducted by the Institute of Natural Resources for assessment purposes.

Samples of additional invertebrates (*Mytilus edulis*, *Chlamys islandica*), fish (*Salvelinus alpinus alpinus*, *Salmo salar*), seabirds (*Uria lomvia*, *Rissa tridactyla*, *Gavia immer*) and marine mammals (*Pagophilus groenlandicus*, *Globicephala melas*, *Balaenoptera acutorostrata*) along with supplementary samples of spottet wolffish (*Anarhichas minor*) and Atlantic wolffish (*Anarhichas lupus*) were collected from local catches taken around Nuuk late summer 2003.

Eider ducks (*Somateria molissima*, *Somateria spectabilis*) were collected at the same location the following winter (2003/2004). Sampling was performed at the landing site in Nuuk immediately after landing of the catch. For wolffish (*Anarhichas minor*, *Anarhichas lupus*) and minke whale samples were purchased from the local market 5-48 hours post-mortem. Samples were taken directly to the Institute of Natural Resources where they were sub-sampled and deep frozen (-50°C).

For all other marine mammals (*Cystophora cristata*, *Delphinapterus leucas*, *Monodon monoceros*, *Odobenus rosmarus*, *Phoca hispida*, *Ursus martini*) taken at different locations and periods (Table 1) sampling was performed immediately after the killing had taken place. Samples were deep frozen (-28°C / -50°C) 6-24 hours post-mortem.

For selected species, e.g. Northern shrimp and snow crab as well as for polar cod, Boreoatlantic armhook squid, Greenland halibut, Greenland cod (*Gadus ogac*), Atlantic cod (*Gadus morhua*) and Atlantic wolffish, the samples were taken separately for males and females or for different size groups. The results for the different sex or size groups were tested for statistical significance applying t-tests or in the case of non-normal data, Mann-Whitney rank sum tests (Sokal and Rohlf 1995). The tests were performed on the derived trophic positions and the data were pooled for subsequent presentation if no significant difference at the 5% level were detected between groups.

### Stable isotope analysis

Samples were prepared for stable carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) isotope analyses at Environmental Canada and analysed in the mass spectrometer laboratory of the Department of Soil Science, University of Saskatchewan, Saskatoon, Canada. Samples were washed in distilled water, freeze-dried, powdered and treated with a 2:1 chloroform-methanol solution to remove lipids. Samples were then dried under a fume hood. Zooplankton were soaked in 0.1 N HCl to remove carbonates, rinsed and then dried. Homogenized samples of 1mg were loaded into tin cups and combusted at 1200°C in a Robo-Prep elemental analyzer. Resultant  $\text{CO}_2$  and  $\text{N}_2$  gases were then analysed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer (CFIRMS), with every five samples separated by two laboratory standards (Bowhead whale baleen and egg albumen). Stable isotope abundances were expressed in the  $\delta$  notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = ((R_{\text{sample}}/R_{\text{standard}}) - 1) * 1000 \quad (1)$$

Where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . The  $R_{\text{standard}}$  values were based on the Pee Dee Belemnite for  $^{13}\text{C}$  and atmospheric  $\text{N}_2$  for  $^{15}\text{N}$ . Replicate measurements of internal laboratory standards indicate measurement errors of  $\pm 0.1\text{‰}$  and  $\pm 0.3\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements respectively.

### Food web model ( $\delta^{15}\text{N}$ )

Derived trophic levels (TL) were calculated according to the approach of Hobson and Welch (1992), Fisk et al. (2001) and Hobson et al. (2002). In brief we assigned the herbivorous copepod *Calanus finmarchicus* (Levinsen et al. 2000) to occupy the second trophic level (i.e. TL=2.0). A theoretic isotopic discrimination factor (TIF) of  $3.8\text{‰}$  was assumed (Hobson and Welch, 1992) and so TL was calculated as:

$$\text{TL} = 2 + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{Calanus finmarchicus}}) / 3.8 \quad (2)$$

Due to the suggested diet-tissue isotopic fractionation factor of  $+2.4\text{‰}$  in birds (Hobson 1993) the following equation was used for sea birds only:

$$\text{TL}_{\text{bird}} = 3 + (\delta^{15}\text{N}_{\text{bird}} - (\delta^{15}\text{N}_{\text{Calanus finmarchicus}} + 2.4)) / 3.8 \quad (3)$$

### Feeding habitats

Stomach contents and feeding habitats were obtained from a literature review focusing specifically on West Greenland waters. For some fish species, the review was guided by searching FishBase (Froese and Pauly 2005).

## RESULTS

### Stable-nitrogen and food web model

Mean  $\delta^{15}\text{N}$  values ranged between  $6.1\text{‰}$ - $20.2\text{‰}$ . The most depleted  $\delta^{15}\text{N}$  value was represented by the suspension feeding blue mussel (*Mytilus edulis*) and the most enriched value by top predator polar bear (*Ursus maritimus*). For the groups of invertebrates, fish, seabirds and marine mammals, mean values ranged from  $6.1\text{‰}$ - $13.1\text{‰}$ ,  $10.0\text{‰}$ - $15.6\text{‰}$ ,  $11.3\text{‰}$ - $14.3\text{‰}$  and  $11.6\text{‰}$ - $20.2\text{‰}$ , respectively (Table 1).

The mean  $\delta^{15}\text{N}$  value for *Calanus finmarchicus*, appointed to represent TL 2.0, was  $7.9\text{‰}$ . From this, derived TL was calculated for other organisms using equation 3 to give a mean range of 1.5-5.2. For the groups of invertebrates, fish, seabirds and marine mammals, mean values ranged between 1.5-3.4, 2.5-4.0, 3.3-4.0 and 3.0-5.2, respectively (Table 1).

A food web model (Fig. 2) was established including all 42 species of which 4 fish species were separated into 2 or 3 size groups (Table 1). Thirty-two out of the 42 species included in this study occupied TL 3

to 4. From this, fish and seabirds occupied much the same TL, however the great Northern diver (*Gavia immer*) was placed higher in the food web adjacent to the ringed seal (*Phoca hispida*). Additionally Northern shrimp, small Boreoatlantic armhook squid and sand lance (*Ammodytes* sp.) were very similar in TL with capelin and juvenile ( $\approx$  age 1) redfish only slightly above. Also quite similar in TL were e.g. minke whale and Atlantic salmon (*Salmo salar*).

It is noteworthy that 'large' polar cod with a length of 12-15 cm showed an almost identical trophic position as 'medium-size' Atlantic cod with a length of 51-64 cm despite the considerable difference in size of the two species (Table 1, Fig. 2).

The trophic level of the one walrus (*Odobenus rosmarus*) taken off Qeqertarsuaq (Area C, Fig. 1) was at an unexpected high of 3.6 compared to animals from further north at Qaanaaq (TL=3.3, n=10, Møller unpublished data) and those from the Northwater Polynya study (TL=3.2).

### Effect of length on $\delta^{15}\text{N}$

The trophic positions of the gadoids studied (Polar cod, Atlantic cod and Greenland cod) and Greenland halibut increased significant (Spearman rank correlation,  $p < 0.001$ ) with the length of the fish (Fig. 3), which illustrates a change in the diet towards higher trophic levels with size and age. This was pronounced for Greenland halibut with an increase of the trophic level from 2.6-2.8 in the smallest size group (10-19cm, age 1) to values between 3.4 and 4.1 in the largest size group (38-55cm,  $\approx$  age 4+). Here the trophic level of the small Greenland halibut showed a remarkably low variability compared to the other species. This was also the case for 'small' Atlantic cod (25-45cm) with trophic positions between 3.1 and 3.6 indicating a difference in diet within this size group.

### Stable-carbon isotopes and feeding habitat

Mean  $\delta^{13}\text{C}$  values ranged between -20.5‰ and -15.8‰. The most depleted  $\delta^{13}\text{C}$  value was represented by *Calanus finmarchicus* and the most enriched by snow crab. For the groups of invertebrates, fish, seabirds and marine mammals, mean values ranged from -20.5 to -15.8‰, -19.7 to -16.4‰, -19.3 to -17.7‰ and -19.5 to -16.8‰, respectively (Table 1).

Benthic feeding organisms such as snow crab, wolffish (*Anarhichas minor*, *Anarhichas lupus*), eider ducks (*Somateria molissima*, *Somateria spectabilis*) were enriched in  $^{13}\text{C}$  contrasting typically pelagic feeding organisms such as capelin, little auk (*Alle alle*) and seals (*Pagophilus groenlandicus*, *Phoca hispida*). Contra dictionary, walrus generally known as benthic feeder showed an unexpected depletion in carbon-13 (-19.5‰). This observation however can be explained by the unexpected high TL (3.6) derived for this species. The relative high TL indicates a deviation from the typical walrus diet and suggests a more pelagic foraging behaviour by this specific individual, now

supported by the pelagic signal indicated by the relative depletion in  $^{13}\text{C}$ .

To investigate  $\delta^{13}\text{C}$  values as an indicator of feeding habitat (i.e. benthic feeders vs. pelagic feeders)  $\delta^{13}\text{C}$  data was plotted against  $\delta^{15}\text{N}$  values and related to information on feeding type, feeding habitat and main diet collected from the literature (Table 2). Several species, in particular fish, do feed both in the pelagic as well as in the benthic habitat. Most of the fish species included in this study were not strictly bottom living and they were therefore classified as demersal. Seven of 19 fish species examined were considered as predominant benthic feeders according to the main food item reported in the literature.

In an overall analysis pelagic feeders ( $n=36$ ) and benthic feeders ( $n=14$ ) separated into two distinct groups with a significant ( $r^2=0.53$ ,  $p<0.001$ ) linear relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for pelagic feeders (Fig. 4) indicating a trophic enrichment effect in  $^{13}\text{C}$ . No significant enrichment effect was found for the benthic feeders ( $r^2 = 0.14$ , n.s.), though a positive correlation was indicated.

When isolated according to taxa (invertebrates, fish, sea birds, marine mammals) an enrichment-effect was significant for pelagic and benthic fish as well as for pelagic seabirds and pelagic marine mammals. A similar effect, though not statistically significant, was indicated for benthic and pelagic invertebrates. Benthic feeding seabirds and marine mammals could not be evaluated due to the small number of species in these respective grouping (Table 3). Notably the enrichment effect (b: slope of regression line, Table 3) for marine mammals was relative large (2.38) compared the other taxonomic groups (1.43-1.76).

## DISCUSSION

We have established an isotope-based food web model for the West Greenland marine ecosystem. Our model suggests a Sub-Arctic marine ecosystem consisting of 5 trophic levels, a finding that is consistent with the model established for the North Water marine food web (Hobson et al., 2002). Similar findings have been reported for the North East Water (Hobson et al., 1995) and the Lancaster Sound food web (Hobson and Welch, 1992).

The derived trophic levels estimated by our model are also generally in good agreement with the North Water model (shown in parentheses), here exemplified by *Calanus hyperboreus* = 2.1 (2.0), *Calanus glacialis* = 2.4 (2.3), *Boreogadus saida* = 3.5 (3.6), *Alle alle* = 3.4 (3.2) and *Monodon monoceros* = 4.2 (Greenland: 4.1). However some marked differences are apparent with in high-level species, such as *Uria lomvia* = 3.4 (4.0), *Odobenus rosmarus* = 3.6 (3.2), *Phoca hispida* = 4.0 (Thule: 4.4, Grise Fjord: 4.6), *Delphinapterus leucas* = 4.1 (Baffin: 4.1, Greenland: 4.4) and *Ursus maritimus* = 5.2 (5.5). The two models based on the North Water and West Greenland suggests that *Uria lomvia*, *Odo-*

*benus. rosmarus*, *Phoca hispida*, *Delphinapterus leucas* and *Ursus maritimus* have region specific diets, but more importantly (apart from *Odobenus rosmarus*) they seem to forage at a lower trophic level in the West Greenland ecosystem. However, our study did also indicate a change in diet towards higher TL with size ( $\approx$  age) for the four fish species investigated. Differences in size and age composition will therefore result in different TL. This can explain differences between the models, but to what degree is uncertain, since comparative data on size is not available from the North Water study. In much the same manner sexual status may be of significance (pregnant/lactating cetaceans may forage on different prey partially due to different habitat choice; e.g. North Atlantic minke whales, cf. for overview Born et al. 2003) and most likely a combined effect is in play. Additionally it should be recalled that, in contrast to the North Water model, we appointed *Calanus finmarchicus* as the TL=2 reference based on its status as a herbivore and a low in  $\delta^{15}\text{N}$  among the copepod species. However, this difference in reference-species only has a minor effect on the derived TL (*Calanus hyperboreus*:  $\Delta\text{TL}=0.1$ ).

Due to a few deviations from the main sampling period of June-September (November-April: 4 seabirds and 6 marine mammals; ultimo-April to primo-June: 3 copepod species), it could be argued that our food web model is a seasonal integrated model representing both a "summer" and a "winter" situation. Our ambition, however, was to establish a food web model assigning trophic levels to key species, also allowing the evaluation of species of migratory behaviour contributing significantly though only seasonally to the West Greenland marine ecosystem. As described all species have been assigned a TL based on the all year round herbivorous *C. finmarchicus* (TL=2.0) and therefore the assigned TL is, disregarding sampling period, an indication of their relative trophic position at that specific time of year (i.e. sampling periode). During the following discussion where general information on diet is related to our food web model, caution has been taken in the interpretation for predator-prey relations when collected at different periods. Additionally stable isotopes in muscle tissue of homeotherms is believed to represent dietary integrations over 1-2 month (Tieszen et al. 1983, Hobson et al., 2002) and is important to keep in mind when addressing highly mobile seabirds and marine mammals that often change region, habitat and feeding habit during the year.

### **Invertebrates**

The model based on the TL values confirms the knowledge about the trophic structure at the base of the pelagic food web. The most important filter feeders harvesting the primary production in the water column (*Calanus*) and from the bottom (*Mytilus*) both have values suggesting that they are primarily herbivores.



## Fish

The bottom-living (demersal) fish species considered in this study do not exclusively feed on benthic organisms, except for a few cases such as wolffish (*Anarhichas lupus* and *A. minor*), shorthorn sculpin (*Myoxocephalus scorpius*) and American plaice (*Hippoglossoides platessoides*). Furthermore, species like Greenland halibut, Polar cod and Atlantic cod as well as Boreoatlantic armhook squid change their diet with increasing size from crustaceans towards a higher proportion of fish (Grunwald 1998, Pedersen and Riget 1993, Kristensen 1984). In general, this is well reflected by the results of the stable isotope analysis with fish feeding species at relative high trophic levels based on the enrichment in  $\delta^{15}\text{N}$  and the allocation of several demersal species in the overlapping area of the pelagic and the benthic component of the food web according to the relationship between the enrichment in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . For sand lance (*Ammodytes* sp.), American plaice and haddock, however, the results of the stable isotope analyses suggest a higher proportion of pelagic food items than found in previous stomach content analyses of American plaice off West Greenland waters (Grunwald 1998) or generalized findings for sand lance and haddock (*Melanogrammus aeglefinus*) from other areas, i.e. the Northwest Atlantic and the Barents Sea, respectively (Bowman et al. 2000, Jiang and Jørgensen 1996).

## Seabirds

Large numbers of common eider (*Somateria molissima*), king eider (*Somateria spectabilis*), thickbilled murres and little auk winter in the West Greenland ecosystem (The Open Water Area) (Merkel et al. 2002, Boertmann et al. 2004) where they were sampled in this study during winter. Although these species represent most of the seabird biomass in West Greenland during winter (Mosbech and Boertmann 2002), they breed in different areas ranging from Canadian Arctic to the eastern Atlantic (Lyngs 2003, Mosbech et al. [submitted](#)) and the numbers of these species in West Greenland (south of Avannarsuaq / North Greenland) during summer is one or several orders of magnitude lower. During summer surface-feeding species like fulmars and gulls are the most numerous seabirds in West Greenland south of Disko Bay.

In this study common eiders and king eiders were sampled in January and at this time of year satellite telemetry shows that the eiders are relatively sedentary/stationary (Mosbech et al. [submitted](#), Merkel et al. [submitted](#)). The king eider and the common eider has low TL values in accordance with their relatively well documented benthic invertebrate diet in West Greenland (see Table 2). Given the low TL of blue mussels an even lower TL could have been expected for common eider if blue mussels were a major food item for common eider as it is in many areas (Goudie et al 2000). However, in Nuuk fjord, where the eiders were sampled, blue mussels were not a dominant food item (Merkel et al. [submitted](#)).

The little auk is known to eat pelagic crustaceans (Table 2). Although stomachs from West Greenland have not been analysed it has a corresponding low TL value of 3.4 (March) and the same level 3.2 as found in the little auk breeding area in the North Water (Hobson et al. 2002). The same low TL value was found in this study for thick-billed murre (November) and kittiwake (September) in contrast to the higher values found in the North Water, 4.0 and 3.9 respectively (Hobson et al. 2002), indicating that these two species were feeding at a lower trophic level preceding sampling in West Greenland. Falk and Durinck (1993) studied stomach contents in thick-billed murre taken by hunters from Nuuk during the winter 1988-89 and found that thick-billed murre fed almost exclusively on fish and euphausiids (*Thysanoessa* sp.) with increasing importance of the latter during winter. In October and December fish accounted for >90% (85% capelin) and 75% respectively of the estimated diet by wet weight. While fish diet accounted for about 40% in November, January and February and only for about 10% in March. The thick-billed murre is an opportunistic feeder taking fish when readily available and the low TL for birds sampled in November in this study indicates that they had been feeding mainly on zooplankton in the preceding month in contrast to the study from 1988-89.

The great Northern diver (*Gavia immer*) breeds in low numbers at lakes in West Greenland and moves to the sea in late August–September (Boertmann 1994). Although migration pattern is largely unknown, apparently most birds migrate south and leave West Greenland for the winter in October while a few winter at sea in the area, immature birds stay at sea year-round and adults may also feed at sea while nesting in a lake.

The TL value of 4.0 for the great northern diver is markedly higher than for the other birds, which range from 3.3 to 3.5. This is well in accordance with the great northern diver taking advantage of its larger size (>4.5 kg) eating mainly large fish – reportedly up to 28 cm. It is known to feed extensively on arctic char in lakes during summer (Cramp 1998), although no stomach analysis data from West Greenland exist.

### **Marine mammals**

Our estimates of trophic level (TL) of the marine mammals based on the  $\delta^{15}\text{N}$  values grouped them minke whales at TL 3 and some that were at TL 4 (ringed seal, beluga and narwhal). Walrus and two seal species were intermediate among these groups: Walrus (TL 3.6), harp seal (*Pagophilus groenlandicus*) (TL 3.6) and hooded seal (*Cystophora cristata*) (4.4). Polar bear was at the highest TL (5.2).

### **Minke whale**

Minke whale was placed at TL 3; i.e. at the same TL as several fish species (e.g. demersal medium-sized Greenland halibut and deep-water redfish and pelagic capelin) and two benthic feeding birds (common eider and king eider).

Minke whales are mainly a summer immigrant to the West Greenland area (e.g. Kapel and Petersen 1982). The body length of the minke whales in our study ranged between 450 and 550 cm. Although age determination in this species is difficult, information in Olsen (2002) indicates that our material consisted of young animals (perhaps < 3 years of age).

The mean  $\delta^{15}\text{N}$  value for six samples included in our study placed minke whales at TL 3.1, similar to the TL (3.1) inferred from  $\delta^{15}\text{N}$  in 43 minke whales taken in West Greenland during May-October 1998 (Born et al. 2003).

We did not have information on the food of the minke whales in 2003. The food preferences of this piscivorous and carcinophagous species may differ regionally and seasonally. However, capelin and sand lance (*Ammodytes* sp.) are important food for minke whales in West Greenland waters. It appears that capelin is the most important prey for minke whales feeding in coastal waters whereas offshore sand lance may be of greater importance; crustaceans can play a significant role in several areas or periods (cf. Neve 200, Andersen et al. 2003 and Born et al. 2003 for reviews). Analysis of stomach contents of 75 minke whales sampled in 1998 in Greenland (<ca. 10 km from shore) revealed that fish was the dominant food item (found in 69% of the stomachs; capelin: 18%, *B. saida*: 5%, *Ammodytes* sp.: 2%, unidentified fish: 44%). Crustaceans (predominantly euphausiids) were present in 25% of the stomachs. A similar menu appeared from reports by the West Greenland hunters on the stomach contents of 141 minke whales caught in 1998 (Born & Dietz unpubl. data).

Hence, minke whales may have different trophic position depending on area and season as also indicated by studies of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in Born et al. (2003) where the TL of minke whales sampled in various areas on the North Atlantic ranged between 2.9 and 3.4. The  $\delta^{13}\text{C}$  values in West Greenland minke whales was generally more depleted than those in minke whales sampled in other areas of the North Atlantic (Born et al. 2003) indicating that they feed relatively near-shore in West Greenland.

### **Harp seal and walrus**

Harp seal together with walrus occupied an intermediate position between TL 3-4 (3.6). A comparison with growth-at-age data (Knutson & Born 1994) indicates that the one walrus in the present study was 3+ years old. Although they sometimes eat vertebrates like seals, birds and fish occasionally, walruses are benthic feeders almost exclusively foraging on bivalves (e.g. Fay 1982, Born et al. 2003).

Walruses in NOW and at West Greenland belong to two genetically and geographically sub-populations (Andersen & Born 2000). However, the little information available on the food of walruses in West Greenland indicates that basically the menu in this area consists of bivalves (*Mya* and *Serripes* Born et al. 1994; *Hiattella*, Born unpubl. data) similar to the situation in the NOW area (Vibe 1950). The present study did not include typical bivalve walrus food items but the

bivalves that were analysed (*M. edulis* and *C. islandica*) were at TL 1.5 and 2.0, respectively. In bivalves from the NOW area including typical walrus food such as *Astarte* sp., *Macoma* sp. and *Yoldia* sp. (Fay 1982, Vibe 1950) the TL ranged between 1.8 and 3.0 (Hobson et al. 2002).

The  $\delta^{13}\text{C}$  value of walrus (-19.5‰) was low compared to the other shallow-water molluscivores: the king eider and the common eider (-17.7‰ and -17.3‰; Table 1).

### **Narwhal, beluga and ringed seal**

The TL of narwhal (*Monodon moneceros*), beluga (*Delphinapterus leucas*) and ringed seals was ca. 4 indicating an overlap in food preference. These marine mammals were placed at the same TL as the demersal fish: “large” Atlantic cod and “large” shorthorn sculpin, and the pelagically feeding bird great Northern diver.

Ringed seal, beluga and narwhal all occur during winter in West Greenland. In spring narwhals and beluga migrate to their northern summering grounds (Heide-Jørgensen 1994). Little is known about the movement in West Greenland of the relatively more “sedentary” ringed seals but likely they disperse during summer to offshore and/or more northern areas as is apparently the case in Northwest Greenland (Kapel et al. 1998). The  $\delta^{13}\text{C}$  value of these three species was very similar. However, a significant difference in body size between ringed seals and the two monodontids likely influence on prey selection (size) and thereby reduce resource competition. Narwhals have different diets than beluga at the wintering grounds (Heide-Jørgensen and Teilmann 1994, Laidre and Heide-Jørgensen 2005) and also prefer to feed at greater depths (Laidre et al. 2004a,b).

In seven narwhals (no age) sampled during November 2000 in the Uummannaq area the  $\delta^{15}\text{N}$  value was 16.1‰ placing them at TL 4.2. The  $\delta^{15}\text{N}$  value in narwhals (N=40) sampled in the same area in November 1993 was 16.4‰ (Dietz et al. 2004) indicating a similar TL. This was also the case with narwhals (N=4) sampled during August in 1993 in the Qaanaaq municipality at the eastern side of the North Water Polynya ( $\delta^{15}\text{N}$ =16.2; TL=4.2). However, lower  $\delta^{15}\text{N}$  values (15.6‰) and (TL 4.0) in narwhals sampled in Qaanaaq during August 1984 and 1985 (Dietz et al. 2004) indicated inter-annual variation in feeding.

Analyses of stomach contents of narwhals indicate both regional and seasonal variation in feeding (Laidre & Heide-Jørgensen 2005). In August 1984 and 1985 the predominant food of narwhals (N=43) in the Qaanaaq area was *B. saida* and *A. glacialis*. However, more than 20% of the stomachs contained *Gonatus fabricii* (Ibid.). In contrast, narwhals that had been taken in the Uummannaq area during mid November (Dietz et al. 2004, Laidre & Heide-Jørgensen 2005) had exclusively been feeding on *G. fabricii* (N=51, Laidre & Heide-Jørgensen 2005). In narwhals sampled during the period December-

April in the Disko Bay area *G. fabricii* was still important. However, Greenland halibut and *Pandalus* sp. constituted a greater fraction of the food (Laidre & Heide-Jørgensen 2005). Hence, depending on seasonal and regional variation in food available, narwhals may be piscivorous and teuthophagous to a varying degree.

Beluga whales sampled in December 2000 in the Vaigat region (northern Disko Island) were aged to an average of 10 GLG's (Growth Layer Groups) (Christina Lockyer, Age Dynamics) (Range:1-42 GLG's) and had a TL of 4.1. Hobson et al. (2002) placed beluga from Baffin Island at TL 4.1, and beluga from West Greenland (ages not stated) at TL 4.4. However, it is not clear from Hobson et al. (2002) where these beluga were sampled except for a statement that they were "from several western Greenland communities" (i.e. apparently outside the NOW area). In the Estuary of the Gulf of St. Lawrence area, the TL of 2+ year-old belugas was 4.6-4.8 in males, and 4.4-4.5 in females (Lesage et al. 2001).

In the Disko bay area belugas consume *B. saida*, *A. glacialis*, redfish (*S. marinus*), Greenland halibut, squid and *Pandalus* sp. (Heide-Jørgensen and Teilmann 1994). Further south they were reported to feed on Atlantic cod, redfish, Greenland halibut and small wolffish (*Anarchicas* sp.) (Degerbøl and Nielsen 1930).

The isotopic values placed narwhal and beluga at the same TL thereby indicating that these similar-sized monodontids compete for food in West Greenland. However, *Gonatus* (TL 2.6; this study) is an important food for narwhals at the wintering ground (Laidre & Heide-Jørgensen 2005) and one would expect narwhals to have a lower TL than the apparently more piscivorous beluga (TL of preferred fish prey 3.0-3.3, this study).

In narwhals  $\delta^{15}\text{N}$  values are relatively high during the first year of life and then show a marked decrease over the first years of life and then a slight increase to a stable level reached around the 10<sup>th</sup> "growth layer age" (Dietz et al. 2004). We did not have any information about the age of the narwhals included in our study. We cannot exclude that the  $\delta^{15}\text{N}$  value and consequent TL in this species may have been affected by age to an unknown degree.

Somewhat surprisingly, the  $\delta^{13}\text{C}$  values of beluga and narwhal were similar (-18.3) indicating that they feed at the same depths during winter. Aerial surveys conducted during March showed that the two species are sympatric along the West Greenland coast between 66°30' and ca. 69°N (Heide-Jørgensen & Acquarone 2002). However, generally beluga winter closer to the coast in West Greenland at relatively shallow depths than do narwhals that mainly winter offshore where they feed at great depths (Heide-Jørgensen 1994, Koski and Davis 1994, Dietz et al. 2004, Laidre et al. 2004a,b) likely on the same species as inshore (Laidre and Heide-Jørgensen 2005).

Competition reduced by narwhals occurring further offshore during winter where they feed on *Gonatus* and Greenland halibut whereas white whales occur closer to shore where they have a broader menu.

### Ringed seal

The TL of ringed seals in our study was 4.0 and represented young animals of  $\leq 2$  yr (the age of 4 specimens were  $\leq 1$  yr based on counting of tooth growth layers; by reference to information in Helle (1992) the remainder were judged to be  $\leq 2$  yr based on standard body length).

The euryphagous ringed seals feed both pelagically and benthically (e.g. Siegstad et al., 1998, Holst et al., 2001). Their food consists of a variety of crustaceans (mainly the hyperiid amphipod *Themisto [Parathemisto] libellula* and fish (mainly polar cod, *Boreogadus saida*, and Arctic cod, *Arctogadus glacialis*) (Ibid.). There are indications that prey selection and feeding strata in the water column differ regionally and among age and sex categories. Immature ringed seals forage at different depths than older (Born et al. 2004) and immature generally take crustaceans in preference of fish (Holst et al. 2001). Even though long-range movement of some individuals has been documented, studies involving tagging and satellite telemetry indicate a large degree of site fidelity in ringed seals in the Baffin Bay-Davis Strait area (Heide-Jørgensen et al. 1992, Kapel et al. 1998, Teilmann et al. 1999, Born et al. 2004).

Although slight differences in  $\delta^{13}\text{C}$  between ringed seals sampled during the late spring to early summer period in the eastern and the western side of the NOW area, respectively, in general the two populations were feeding at the same trophic level, based on  $\delta^{15}\text{N}$ , and essentially on the same food items, especially *B. saida* and *A. glacialis* (Holst et al. 2001). Analyses of stomach content showed that *B. saida* were the most important prey (87 weight %) in the Qaanaaq area (Siegstad et al. 1998).

Based on  $\delta^{15}\text{N}$ , ringed seals in the NOW area had a TL of 4.4 and 4.6 with indications of a regional difference (highest in west) (Hobson et al. 2002). TL in a sub-sample of 8-10 year old (i.e. adult) ringed seals in Hobson et al. (2002) was 4.6 (Campbell et al. 2005).

Adult *B. saida* in the NOW were at TL 3.6 ( $\delta^{15}\text{N}=14.2$ ) (Hobson et al. 2002), similar to medium-sized (12-15 cm) *B. saida* in the present study (TL 3.5,  $\delta^{15}\text{N}=13.7\text{‰}$ ).  $\delta^{15}\text{N}$  in large and small *B. saida* in Lancaster Sound was 15.2‰ and 11.1‰, respectively (Hobson & Welch 1992). In the Gulf of St. Lawrence  $\delta^{15}\text{N}$  of *B. saida* was 14.0 placing them at TL 3.8 (Lesage et al. 2001). In the NOW area *Themisto libellula* were at TL 2.5 ( $\delta^{15}\text{N}=9.7\text{‰}$ ) (Hobson et al. 2002),

The relatively low TL in ringed seals in our study indicates that they generally forage at a lower trophic level in West Greenland compared to the NOW area. This assumption is not supported by analyses of stomach contents. During spring ringed seals in the Disko Is-

land/Qeqertarsuaq area mainly feeding on capelin and redfish (*Sebastes* sp.) (ca. 77% by weight) with *Thysanoessa* making up ca. 17% of the food (Siegstad 1998). In the neighbouring Kangaatsiaq area the spring diet consists mainly of *Gonatus* (ca. 46%), *Gadus* sp. (ca. 21%) and *B. saida* (ca. 13%) (*Ibid.*). The TL of these species ranges between 3.0 and 3.5 (this study).

However, a comparison of regional differences in trophic niches based on stable isotopes is sensitive to differences in sex and age of the samples. The relatively low TL of ringed seals in our study might be explained by the fact that our sample consisted of immature seals. Generally, this age group forage on crustaceans (e.g. Holst et al. 2001) with a lower TL than fish.

In our study,  $\delta^{13}\text{C}$  was  $-18.8\text{‰}$  compared to  $-19.4\text{‰}$  (Qaanaaq) and  $-18.3\text{‰}$  (Jones Sound) in the NOW (Hobson et al. 2002), and  $-18.0\text{‰}$  (Campbell et al. 2005). Ringed seals collected in Jones Sound had significantly higher  $\delta^{13}\text{C}$  values than those collected in the Qaanaaq area suggesting that they took more benthic or inshore prey (Holst et al. 2001). The  $\delta^{13}\text{C}$  value in the present study may indicate that ringed seals in the Disko Bay area select a feeding habitat (stratum) that is not different from that in the Qaanaaq area where immature ringed seals generally exploit the upper 50 m of the water column in contrast to older animals that dive deeper (Born et al. 2004).

The asymptotic body mass of adult ringed seals is 50-75 kg (Lydersen 1998, Kingsley 1998) whereas that of narwhal and beluga is 700-1350+ kg (Stewart 1994, Heide-Jørgensen & Teilmann 1994, Laidre et al. 2004a ). A 2-fold difference in mass, or 1.25 ratio if using linear dimensions, is considered sufficient for competition avoidance (Schoener 1974, Bowers & Brown 1982). Likely, competition at the wintering grounds between narwhals and white whales on one side and ringed seals on the other is reduced because of difference in body size and consequent differences in prey size, and diet composition. Furthermore, competition is reduced because ringed seals primarily winter in the fast ice habitat (e.g. Born et al. 2004 for a review) where the two odontids rarely occur (Heide-Jørgensen 1994).

### **Harp seal and hooded seal**

The highly migratory (Sergeant 1976) harp seal and hooded seal had TLs (3.6 and 4.4, respectively) that were intermediate between the two major groups of marine mammal TLs in our study. Harp and hooded seals are migrants to West Greenlandic waters usually occurring there during the "summer" or "open water period" (e.g. Kapel & Petersen 1982). Inferred from body lengths (Innes et al. 1981), the harp seals in our study were <3 years of age. Using the same method (Wiig 1985), the hooded seals were probably 5+ years old (the lengths of the hooded seals in our table are implausible).

The diet of harp seals in West Greenlandic waters varies both regionally and seasonally (Kapel 2000). Capelin is by far the most important food item in Southwest and Central West Greenland. In Southwest Greenland, fish constituted ca. 87% of the diet by weight (76% cape-

lin) of harp seals caught inshore during August-November; Northern shrimp (*Pandalus borealis*) and krill (euphausiids) made up ca. 12% (*Ibid.*). Apparently, the diet offshore is different. In harps seals taken during August offshore in Southwest Greenland, other fish (ca. 65% by weight; in particular *Ammodytes*) made up the bulk of the food, and crustaceans (*Parathemisto*, *Pandalus*) constituted ca. 30% (Kapel 2000). Kapel (2000) suggested that apparently the diet of harp seals in West Greenland depends on the season and locality, rather than on the age of the seals. This is in accordance with Lesage et al. (2001) who, despite an indication of yearlings feeding at lower trophic levels than adults, did not find any significant differences in TL among sex and age groups of harp seals in the Gulf of St. Lawrence area. The TL of harp seals in the study by Lesage et al. (2001) varied between 3.8 and 4.7 depending on year of sampling and area (estuary vs. gulf). We do not have any information about whether the harps seals in the present study had been taken by the hunters inshore or offshore and it cannot be excluded that the relatively low TL of these seals can be attributed to the fact that they primarily had been feeding offshore on krill. The  $^{13}\text{C}$  enrichment was at the same level as that in the relatively coastal ringed seal.

Based on the  $\delta^{15}\text{N}$  value hooded seals was at TL 4.4. Analysis of a few stomachs indicated that capelin was the most important prey (ca. 93% by weight) in Southwest Greenland (Kapel 2000). Similarly, reports from hunters from their spring hunt indicated that fish make up the bulk of food (ca. 97% of 828 stomachs) in this order of falling importance: Cod sp., redfish sp. and capelin. Crustaceans and squid were only reported in <2% and <1% of the stomachs, respectively (*Ibid.*). In the Gulf of St. Lawrence, 2+ hooded seals were at the highest trophic level (4.7-5.0). Greenland halibut and *B. saida* are the two most important prey of hooded seals off Newfoundland during winter (Ross 1993). If the signal of diet from  $\delta^{15}\text{N}$  is retained for up to 2 months, then the  $\delta^{15}\text{N}$  value in hooded seals landed in West Greenland may reflect the feeding at the wintering ground. Hooded seals that are caught during spring in Southwest Greenland are immigrants from the Newfoundland area (Kapel 1982 and references therein, Hammill 1993). Next to polar bear, hooded seal was the most  $^{13}\text{C}$  enriched species in the present study indicating that it is a benthic feeder.

Polar bear (1 specimen) had the highest TL (5.2) in this study. Judged from the body length (Derocher & Stirling 1998) this bear was adult. This is in accordance with Hobson et al. (2002) where polar bears from Lancaster Sound were at TL 5.5. Polar bear mainly feed on ringed seals although bearded seal (*Erignathus barbatus*) and other vertebrates are also eaten (reviewed in Hobson et al. 2002). We do not have any specific information about the prey of polar bears in Central West Greenland. However, bears in this area belong to the Baffin Bay sub-population (Taylor et al. 2001). Ringed seals are widely distributed in the Baffin Bay pack is (Finley et al. 1983). If the polar bear had been feeding on ringed seals its lower TL compared to that in Hobson et al. (2002) may be explained by the fact that the TL of ringed seals in our study was lower than that in ringed seals in Hobson et al. (2002).



Our study did not include several species of marine mammal that are known to occur regularly in West Greenland. Some of these seals and whales occur there year-round (bearded seal, *Erignathus barbatus*, harbour seal, *Phoca vitulina*); some only during winter (bowhead whale, *Balaena mysticetus*) and some mainly during summer (humpback whale, *Megaptera novaeanglia*, fin whale, *Balaenoptera*<sup>17</sup> *physalus*, blue whale, *Balaenoptera musculus*, sei whale, *Balaenoptera borealis*, killer whale, *Orcinus orca*, harbour porpoise, *Phocoena phocoena*, white-beaked dolphin, *Lagorhynchus albirostris*, white-sided dolphin, *Lagorhynchus acutus*) (cf. Born 2001).

We present a food web-model for the West Greenland marine ecosystem integrated over the entire year. However, our sampling periods basically represented a winter and a summer situation. All invertebrates, fish and shark, and some birds (kittiwake, great northern diver) and marine mammals (long-finned pilot whale, minke whale and harp seal) were sampled during the summer or “open water season”. In contrast, those birds (common eider, king eider, little auk, brünnichs guillemot) and marine mammals (walrus, ringed seal, beluga, narwhal and hooded seal) that were sampled during November-May basically occupy the West Greenland study area only during winter. Harp seal and hooded seal are mainly summer immigrants but also winter in West Greenland in appreciable numbers (Born 2001).

#### **N-fixation mechanism and possible effect on the model**

Our model produced a TL of 1.5 for blue mussel, which was clearly below that expected for a filter feeder. This was likely due to the fact that our model was inappropriate for some organisms that void nitrogenous wastes in different ways. As reviewed by Vanderklift and Ponsard (2003), molluscs and detritivores show the lowest diet-tissue isotopic discrimination for  $\delta^{15}\text{N}$ . This is related to their excretion of primarily ammonia. Such lower discrimination would result in lower tissue  $\delta^{15}\text{N}$  values and hence lower TL estimates. Future refinements of marine TL models using  $\delta^{15}\text{N}$  values should consider the form of nitrogenous excretion and the relative dependence of some organisms on detritus (Vanderklift and Ponsard 2003). Nonetheless, overall, our model performed well describing a 5 TL system as expected.

#### **Carbon-13 and feeding habitat**

The linear relationship identified for  $^{15}\text{N}$  and  $^{13}\text{C}$  values among pelagic feeders indicates close coupling between sources of carbon and nitrogen in this marine foodweb, an effect commonly seen in isotopic datasets of marine consumers (Hobson et al 2002). Apart from invertebrates a similar positive linear relationship was apparent for pelagic feeders within taxa and for benthic feeding fish. Differences in the slope of the  $\delta^{15}\text{N}$  vs.  $\delta^{13}\text{C}$  relationship can be driven by differences in the trophic enrichment effect that undoubtedly occurs among such diverse taxa.

### Implications and future perspectives

Future effort should be made to expand and strengthen stable isotope models like that of the West Greenland marine ecosystem. We recommend a holistic approach where more species and especially those representing lower trophic levels (i.e. invertebrates) are included, as their role in marine ecosystems seem to have been overlooked. A recent example is the amphipod *Themisto libellula* that through SI modelling has been assigned as a key-species in energy-transfer from low-level to high-level consumers in a sub-Arctic system (Hobson et al. 2002). Likewise organisms involved in the microbial-loop are potential key-species in marine Arctic ecosystems (Levinsen and Nielsen 2002) and should therefore be considered. However, aiming to include small and/or C- or N-depleted organisms mostly means having to pool individuals and even so the analysis may not be possible. This dilemma calls for the development of analytical techniques where less C and N is needed for individual analysis.

Based on our own results we recommend careful designed collection schemes where standard biological data (i.e. total length, age, sexual status) are recorded, and this in order to allow for a full interpretation with in and between regions (i.e. models). Obviously sample size needs to be considered in order to assign diet effects based on biological data.

The now established stable isotope model for ecosystem West Greenland is considered as a template to guide future modelling of energy and contaminant flow. Generally carbon and energy flux models rely on the quantification of daily energy requirements and are the combined to estimate total energy flux and to yield energy flux per unit area. In order to convert energy-flux to carbon-flux good information on diet or trophic level is needed and how these vary with season, age, sexual- and reproductive status. Most importantly is the low conversion efficiency between trophic levels i.e. energy-flux through one prey-trophic level is not equivalent to the same energy flux through a higher trophic level. From this it is clear that estimates of trophic position are crucial for accurate estimates of carbon-flux.

On a similar basis the model could assist in investigations of bioaccumulating and biomagnifying of persistent contaminants (Broman et al., 1992; Rolff et al., 1993; Atwell et al., 1998; Hobson et al., 2002; Campbell et al., 2005). In fact, such work is currently being conducted and looking at food web specific bioaccumulation of mercury and methyle-mercury using the West Greenland stable isotope model and fatty acid biomarkers.

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Table 1: Species studied, stable isotope compositions ( $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ ) and derived trophic levels (TL) in the West Greenland marine ecosystem (mean values and standard deviation given for stable isotopes and trophic levels; n: number of samples; \*: see Fig. 1, \*\*: 67°00.5'N, 53°44.8'W; CL: carapace length, CW: carapace width, ML: mantle length, TL: total length, ST: soft tissue)

Common Name	Scientific name	Sampling location *	Sampling period	Year	Size range	n	d13C	d15N	TP
<b>Invertebrates</b>									
Blue mussel	<i>Mytilus edulis</i>	E	Sept.	2003	1.6 - 6.1 g ST	12	-18.3 ± 0.4	6.1 ± 0.5	1.5 ± 0.1
Iceland scallop	<i>Chlamys islandica</i>	D**	July	2003	32.6 - 64.1 g ST	12	-16.9 ± 0.3	7.8 ± 0.2	2.0 ± 0.1
Copepod	<i>Calanus finmarchicus</i>	C	April - May	2005	3.4 - 3.6 mm TL	9	-20.5 ± 0.3	7.9 ± 0.3	2.0 ± 0.1
Copepod	<i>Calanus hyperboreus</i>	C	April - May	2005	10.0 - 10.6 mm TL	10	-19.4 ± 0.7	8.4 ± 0.3	2.1 ± 0.1
Northern Krill	<i>Meganyctiphanes norvegica</i>	D	July	2003	22 - 35 mm TL	2	-19.0 ± 0.0	8.5 ± 0.4	2.2 ± 0.1
Copepod	<i>Calanus glacialis</i>	C	May - June	2005	3.5 - 3.6 mm TL	4	-19.2 ± 0.6	9.2 ± 0.1	2.4 ± 0.0
Northern shrimp	<i>Pandalus borealis</i>	D	July	2003	19 - 29 mm CL	24	-17.4 ± 0.5	10.0 ± 1.8	2.6 ± 0.5
Boreoatlantic armhook squid - small	<i>Gonatus fabricii</i>	D	July	2003	5 - 10 cm ML	12	-18.1 ± 0.3	10.4 ± 0.5	2.6 ± 0.1
Snow crab	<i>Chionoecetes opilio</i>	D	Sept.	2003	62 - 128 mm CW	24	-15.8 ± 0.3	11.0 ± 0.4	2.8 ± 0.1
Boreoatlantic armhook squid - large	<i>Gonatus fabricii</i>	D	Aug. - Sept.	2003	> 10 cm ML	26	-18.8 ± 0.5	13.1 ± 1.7	3.4 ± 0.5
<b>Fish</b>									
Sand lance	<i>Ammodytes sp.</i>	D	July	2003	14 - 22 cm TL	11	-18.8 ± 0.3	10.0 ± 0.3	2.5 ± 0.1
Redfish sp. - juvenile	<i>Sebastes sp.</i>	D	July	2003	8 - 11 cm TL	12	-19.1 ± 0.2	10.5 ± 0.4	2.7 ± 0.1
Greenland halibut - small	<i>Reinhardtius hippoglossoides</i>	D	Aug. - Sept.	2003	16 - 19 mm TL	11	-19.4 ± 0.2	10.5 ± 0.3	2.7 ± 0.1
Capelin	<i>Mallotus villosus</i>	D	July	2003	13 - 16 cm TL	12	-19.0 ± 0.6	11.6 ± 1.2	3.0 ± 0.3
Great silver smelt	<i>Argentina silus</i>	D	July	2003	26 cm TL	1	-17.4	11.8	3.0
Greenland halibut - medium	<i>Reinhardtius hippoglossoides</i>	D	Aug. - Sept.	2003	27 - 32 cm TL	10	-19.3 ± 0.5	11.9 ± 1.0	3.0 ± 0.3
Atlantic salmon	<i>Salmo salar</i>	E	Aug. - Sept.	2003	59 - 64 cm TL	16	-19.7 ± 0.6	12.1 ± 1.1	3.1 ± 0.3
Polar cod - small	<i>Boreogadus saida</i>	D	Aug. - Sept.	2003	8 - 10 cm TL	12	-18.8 ± 0.3	12.3 ± 0.7	3.2 ± 0.2
Deepwater redfish	<i>Sebastes mentella</i>	D	July	2003	21 - 55 cm TL	12	-19.1 ± 0.5	12.6 ± 1.5	3.2 ± 0.4
Golden redfish	<i>Sebastes marinus</i>	D	July	2003	28 - 45 cm TL	12	-18.7 ± 0.6	12.9 ± 1.2	3.3 ± 0.3
American plaice	<i>Hippoglossoides platessoides</i>	D	July	2003	21 - 32 cm TL	12	-18.0 ± 0.4	12.9 ± 0.7	3.3 ± 0.2
Haddock	<i>Melanogrammus aeglefinus</i>	D	July	2003	18 - 24 cm TL	12	-18.0 ± 0.3	13.0 ± 0.5	3.3 ± 0.1
Atlantic cod - small	<i>Gadus morhua</i>	D	July - Sept.	2003	26 - 46 cm TL	13	-18.1 ± 0.3	13.1 ± 0.6	3.4 ± 0.2
Atlantic cod - medium	<i>Gadus morhua</i>	D	July - Sept.	2003	51 - 64 cm TL	25	-18.1 ±	13.1 ± 0.6	3.4 ± 0.2
Arctic char	<i>Salvelinus alpinus alpinus</i>	E	Aug. - Sept.	2003	31 - 39 cm TL	12	-18.3 ± 0.5	13.2 ± 0.4	3.4 ± 0.1
Daubed shanny	<i>Leptoclinus maculatus</i>	D	July	2003	13 - 20 cm TL	12	-17.3 ± 0.5	13.3 ± 0.6	3.4 ± 0.2
Polar cod - medium	<i>Boreogadus saida</i>	D	Aug. - Sept.	2003	12 - 15 cm TL	9	-18.9 ± 0.4	13.7 ± 0.5	3.5 ± 0.1
Greenland cod - small	<i>Gadus ogac</i>	D	July - Sept.	2003	26 - 38 cm TL	7	-17.3 ± 0.2	13.7 ± 0.4	3.5 ± 0.2
Atlantic wolffish	<i>Anarhichas lupus</i>	D, E	July - Sept.	2003	35 - 64 cm TL	12	-17.0 ± 1.2	13.8 ± 1.2	3.6 ± 0.3
Greenland halibut - large	<i>Reinhardtius hippoglossoides</i>	D, E	Aug. - Sept.	2003	38 - 55 cm TL	13	-18.4 ± 0.9	13.9 ± 1.0	3.6 ± 0.3
Spottet wolffish	<i>Anarhichas minor</i>	D, E	July - Sept.	2003	29 - 88 cm TL	13	-16.4 ± 0.9	14.2 ± 0.6	3.7 ± 0.2
Greenland cod - large	<i>Gadus ogac</i>	D	July - Sept.	2003	46 - 60 cm TL	4	-17.5 ± 0.4	15.2 ± 0.6	3.9 ± 0.2
Atlantic cod - large	<i>Gadus morhua</i>	D	July - Sept.	2003	66 - 70 cm TL	4	-17.3 ± 0.7	15.5 ± 0.6	4.0 ± 0.2
Shorthorn sculpin	<i>Myoxocephalus scorpius</i>	C, D	June, July	2003	30 - 40 cm TL	16	-16.4 ± 0.6	15.6 ± 0.8	4.0 ± 0.2
<b>Sharks</b>									
Greenland shark	<i>Somniosus microcephalus</i>	D	July	2003	appr. 400 cm TL	1	-19.5	17.0	4.4
<b>Seabirds</b>									
King eider	<i>Somateria spectabilis</i>	E	Jan	2004	1.52 - 1.83 kg	9	-17.7 ± 0.3	11.3 ± 0.3	3.3 ± 0.1
Common eider	<i>Somateria mollissima</i>	E	Jan	2004	1.48 - 2.16 kg	32	-17.3 ± 0.8	11.4 ± 0.6	3.3 ± 0.2
Little auk	<i>Alle alle</i>	E	March	2003	0.15 - 0.22 kg	19	-19.5 ± 0.3	11.7 ± 0.4	3.4 ± 0.1
Brünnichs guillemot	<i>Uria lomvia</i>	E	Nov.	2003	0.85 - 1.03 kg	12	-19.3 ± 0.4	12.0 ± 0.5	3.4 ± 0.1
Kittiwake	<i>Rissa tridactyla</i>	E	Sept.	2003	0.33 - 0.45 kg	12	-19.5 ± 0.4	12.1 ± 0.8	3.5 ± 0.2
Great Northern Diver	<i>Gavia immer</i>	E	Aug. - Sept.	2003	4.50 - 4.91 kg	2	-17.7 ± 0.5	14.3 ± 0.3	4.0 ± 0.1
<b>Marine mammals</b>									
Minke whale	<i>Balaenoptera acutorostrata</i>	D	Sept.	2003	appr. 450 - 550 cm TL	6	-19.0 ± 1.1	12.0 ± 0.5	3.1 ± 0.1
Harp seal	<i>Pagophilus groenlandicus</i>	E	Aug. - Sept.	2003	100 - 140 cm TL	10	-18.6 ± 0.2	13.8 ± 0.8	3.6 ± 0.2
Walrus	<i>Odobenus rosmarus</i>	C	Jan	2002	250 cm TL	1	-19.5	14.0	3.6
Ringed seal	<i>Phoca hispida</i>	C	April	2003	91 - 108 cm TL	10	-18.8 ± 0.2	15.4 ± 0.4	4.0 ± 0.1
Beluga	<i>Delphinapterus leucas</i>	B	Dec.	2000	292 - 487 cm TL	18	-18.3 ± 0.3	15.9 ± 0.4	4.1 ± 0.1
Narwhal	<i>Monodon monoceros</i>	A	Nov.	2000	---	7	-18.3 ± 0.3	16.1 ± 0.3	4.2 ± 0.1
Hooded seal	<i>Cystophora cristata</i>	E	April - May	2003, 2004	240 - 386 cm TL	10	-16.8 ± 0.3	16.9 ± 0.8	4.4 ± 0.2
Polar bear	<i>Ursus maritimus</i>	C	March	2002	appr. 220 cm TL	1	-16.8	20.2	5.2

**Table 2:** Feeding type, habitat and main diet of West Greenland food web components (DF: detritus feeder, PR: predator, SUS: Suspension feeder, SC: scavenger; juv. juvenile)

Taxa - Scientific name	Feeding type	Feeding habitat	Main food	Reference
<b>Invertebrates</b>				
<i>Mytilus edulis</i>	SUS	benthic	phytoplankton	
<i>Chlamys islandica</i>	SUS	benthic	phytoplankton	
<i>Calanus finmarchicus</i>	SUS	pelagic	phyto- and microzooplankton	Levinsen et al. (2000)
<i>Calanus hyperboreus</i>	SUS	pelagic	phyto- and microzooplankton	Levinsen et al. (2000)
<i>Calanus glacialis</i>	SUS	pelagic	phyto- and microzooplankton	Levinsen et al. (2000)
<i>Meganyctiphanes norvegica</i>	SUS	pelagic	zooplankton (copepods), phytoplankton	McClatchie (1985), Båmstedt & Karlson (1998)
<i>Pandalus borealis</i>	PR, SC, DF	demersal, pelagic	polychaetes, crustaceans (mysids), detritus	Shumway et al. (1985), Bergstroem (2000)
<i>Chionoecetes opilio</i>	PR, SC	benthic	crustaceans (shrimp), fish (capelin), polychaetes, clams	Squires & Dawe (2003)
<i>Gonatus fabricii</i>	PR	pelagic	zooplankton, pelagic fish (e.g. capelin)	Kristensen (1984)
<b>Fish</b>				
<i>Ammodytes sp.</i>	PR	demersal	benthic crustaceans	Bowman et al. (2000)
<i>Sebastes sp.</i>	PR	pelagic, demersal	planktonic crustaceans (euphausiids, hyperiids)	Konchina (1986), Pedersen & Riget (1993)
<i>Sebastes mentella</i>	PR	demersal	planktonic crustaceans (euphausiids), squid and fish (e.g. capelin)	Konchina (1986), Pedersen & Riget (1993)
<i>Sebastes marinus</i>	PR	demersal	planktonic crustaceans (euphausiids), squid and fish (e.g. capelin)	Pedersen & Riget (1993)
<i>Reinhardtius hippoglossoides</i>	PR	demersal	crustaceans (shrimp, mysids), fish (juvenile redfish, polar cod, capelin)	Smidt (1969), Grunwald (1998), Pedersen & Riget (1993)
<i>Mallotus villosus</i>	PR	pelagic	planktonic and benthic crustaceans (Calanus sp., euphausiids, amphipods)	Kleist (1988), Astthorson & Gislason (1997)
<i>Argentina silus</i>	PR	demersal	planktonic crustaceans (euphausiids, i.e. Meganyctiphanes norvegica)	Bowman et al. (2000)
<i>Salmo salar</i>	PR	demersal	squid, shrimp, fish	Maitland & Campbell (1992)
<i>Boreogadus saida</i>	PR	demersal, pelagic	epibenthic mysids, amphipods, copepods, fish (e.g. capelin)	Craig et al. (1982), Jensen (1992)
<i>Hippoglossoides platessoides</i>	PR	demersal	benthic crustaceans, echinoderms, polychaetes, fish	Grunwald (1998)
<i>Melanogrammus aeglefinus</i>	PR	demersal	benthic crustaceans, echinoderms, polychaetes	Jiang & Jørgensen (1996)
<i>Gadus morhua</i>	PR	demersal	crustaceans (shrimp, amphipods, euphausiids), fish (capelin, juv. redfish)	Grunwald (1998)
<i>Salvelinus alpinus alpinus</i>	PR	demersal	planktonic and benthic crustaceans, fish	Morton (1982), Rikarden et al. (2000)
<i>Leptoclinus maculatus</i>	PR	demersal	polychaetes and benthic crustaceans	Makushok (1986)
<i>Gadus ogac</i>	PR	demersal	fish (capelin) and benthic invetebrates	Nielsen & Andersen (2001)
<i>Anarhichas lupus</i>	PR	demersal	brittle stars and sea urchins, hard-shelled mollusks	Grunwald (1998), Ortova et al. (1990)
<i>Anarhichas minor</i>	PR	demersal	sea urchins and other echinoderms, fish (e.g. cod)	Grunwald (1998), Ortova et al. (1990)
<i>Myoxocephalus scorpius</i>	PR	demersal	demersal fish and benthic crustaceans	Gibson & Robb (1996)
<b>Shark</b>				
<i>Somniosus microcephalus</i>	PR	demersal	fish, squid, seals, marine birds	Cortes (1999)
<b>Sea birds</b>				
<i>Somateria spectabilis</i>	PR	benthic (<50m)	bivalves (Hiatella, Macoma, Mya) sea urchins, polychaetes and benthic crustaceans	Frimer (1997), Mosbech (in press)
<i>Somateria molissima</i>	PR	benthic (<20 m)	bivalves (Mya, Mytilus, Hiatella) polychaetes (Pectinaria spp.) and benthic crustaceans	Merkel et al. (in press)
<i>Alle alle</i>	PR	pelagic (upper 20 m)	pelagic crustaceans (copepods, amphipods)	Montevecchi & Stenhouse (2002), Pedersen & Falk (2001)
<i>Uria lomvia</i>	PR	pelagic (upper 50 m)	fish (capelin), pelagic crustaceans (euphausiids)	Falk & Durinck (1993)
<i>Rissa tridactyla</i>	PR	pelagic (upper 1 m)	fish and pelagic invertebrates (crustaceans, analids)	Baird (1994)
<i>Gavia immer</i>	PR	pelagic (upper 10 m)	fish, crustaceans	Cramp (1998)
<b>Marine mammals</b>				
<i>Balaenoptera acutorostrata</i>	PR	pelagic	fish (capelin, polar cod, sand lance) and crustaceans (euphausiids)	Larsen & Kapel (1981), Neve (2001), Born (pers. comm.)
<i>Odobenus rosmarus</i>	PR	benthic (<50m)	bivalves ( <i>Mya</i> , <i>Hiatella</i> , <i>Serripes</i> ) and other benthic invertbrates, fish (sand lance) and seals	Vibe (1950), Lowry & Fay (1984), Born et al. (1994, 2003)
<i>Pagophilus groenlandicus</i>	PR	pelagic	fish (capelin, Atlantic cod) and pelagic crustaceans (shrim, euphausiids)	Kapel (2000)
<i>Phoca hispida</i>	PR	pelagic, benthic (demersal)	fish (capelin, red fish) and pelagic crustaceans (thysanoessa, euphausiids)	Siegstad et al. (1998)
<i>Delphinapterus leucas</i>	PR	pelagic, demersal, bathypelagic	fish (Arctic cod, polar cod, red fish, Greenland halibut), squid and crustaceans	Heide-Jørgensen & Teilmann (1994)
<i>Monodon monoceros</i>	PR	pelagic, bathypelagic	squid ( <i>Gonatus fabricii</i> )	Laidre & Heide-Jørgensen (2005)
<i>Cystophora cristata</i>	PR	bathypelagic, pelagic	fish (capelin) and crustaceans	Kapel (2000)
<i>Ursus maritimus</i>	PR, SC	pelagic, (on ice)	seals (ringed seal), other marine mammals, and scavenging on larger marine mammals	Smith (1980), Stirling & Øritsland (1995), Derocher et al. (2002)

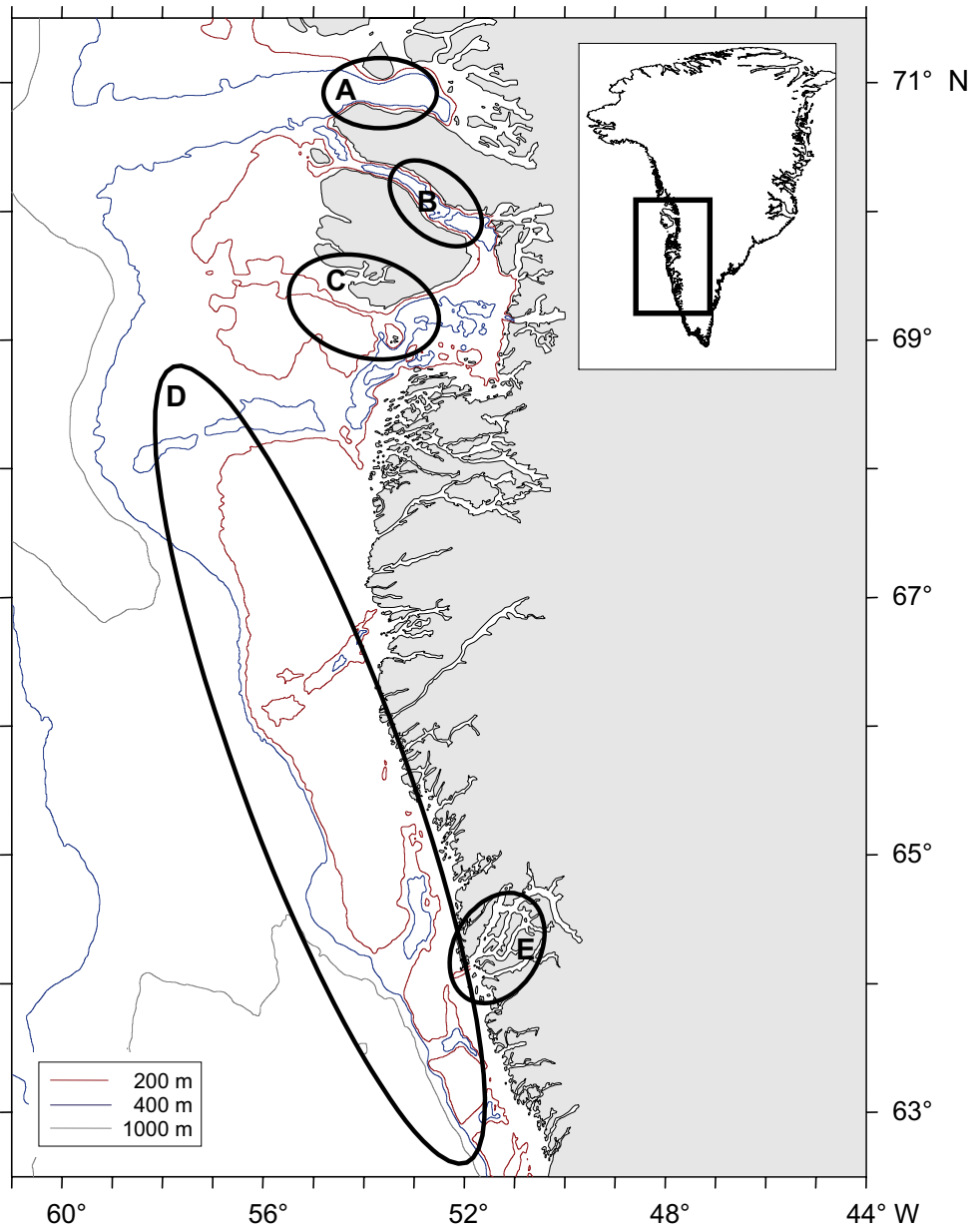


Fig. 1: Study area and sampling locations (A: Ummanaq, B: Vaigat / Saqqaq, C: Disko Bay / Qeqertarsuaq, D: offshore and coastal waters, E: Nuuk fjord area).



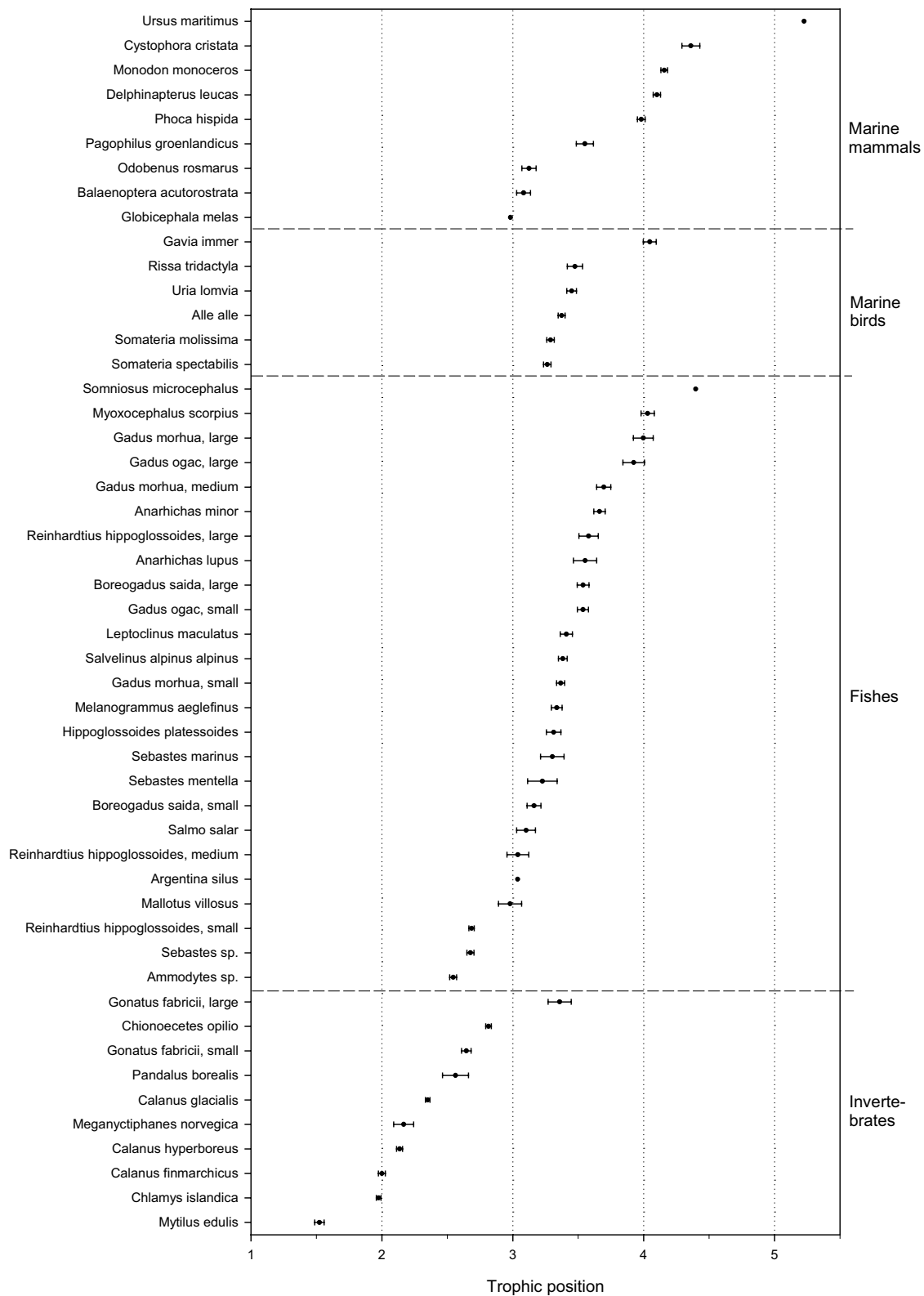


Fig. 2: Food web model for the West Greenland ecosystem based on  $\delta^{15}\text{N}$  (Mean $\pm$ SE). Species with in groups (invertebrates, fish, marine birds and marine mammals) are sorted in ascending order according to trophic level (TL).

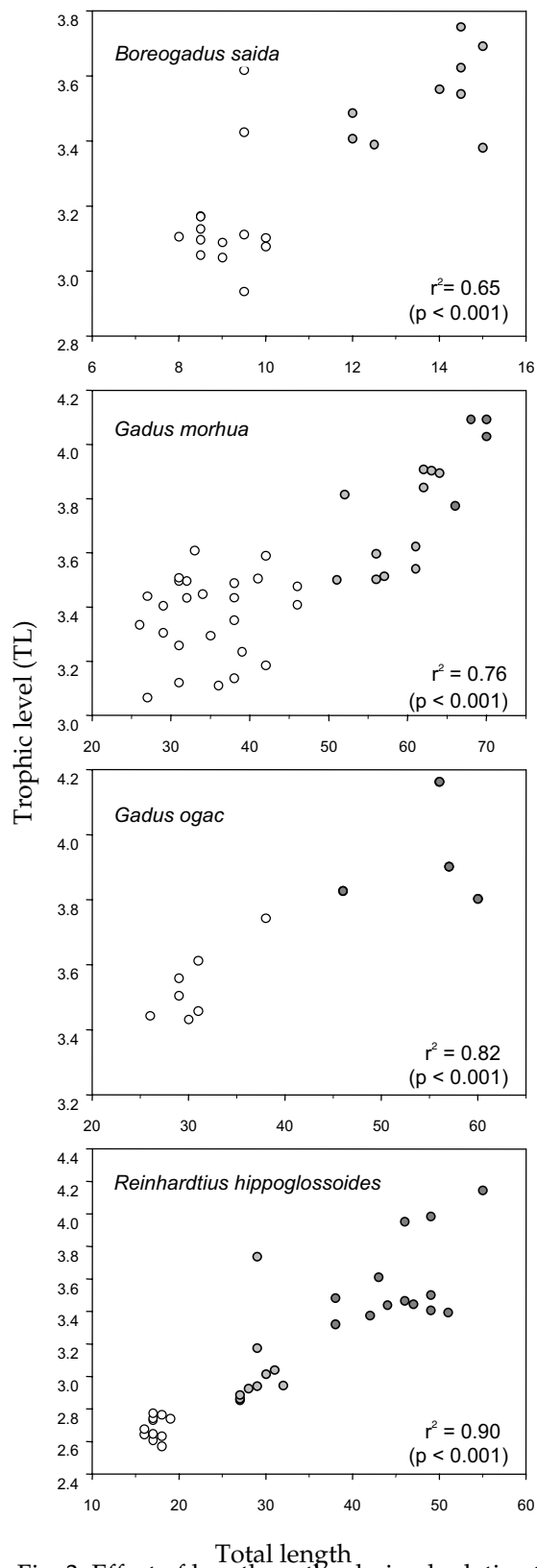


Fig. 3: Effect of length on the derived relative trophic level (TL) for four fish species (shading of the symbols denotes size grouping into small, medium and large as used in Tab. 1,  $r_s$ : Spearman rank correlation coefficient).

## Carbon-13 and Feeding Habitat

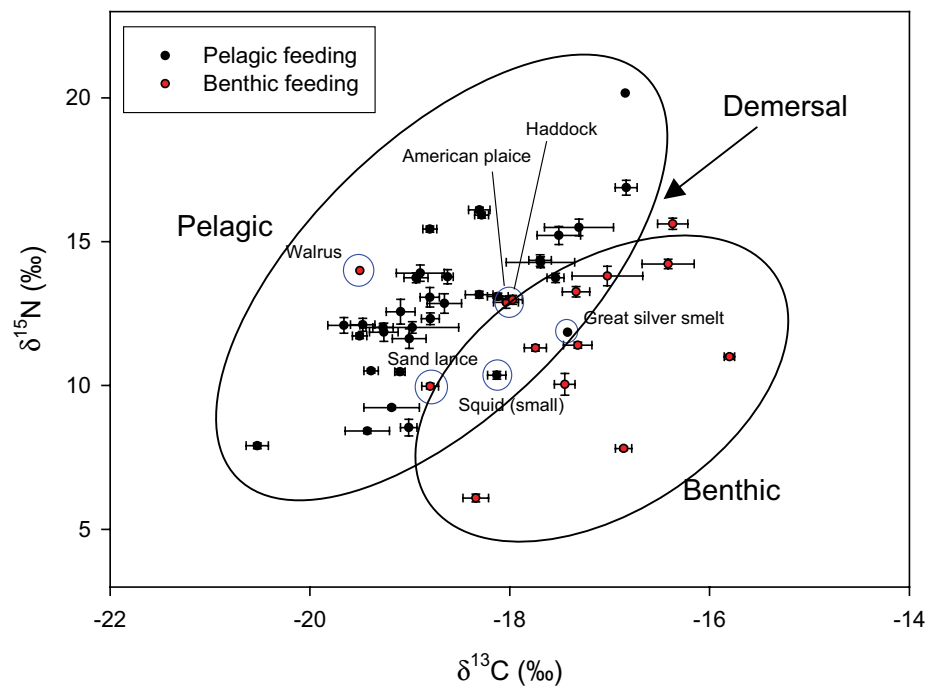


Fig. 4: Pelagic, benthic and demersal components of the West Greenland food web based on stable-carbon and stable-nitrogen (Mean $\pm$ SE). Circled and named species are deviating from the expected component assignment.

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# Regional differences in fatty acid composition in common minke whales (*Balaenoptera acutorostrata*) from the North Atlantic

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## ABSTRACT

Variation in fatty acid (FA) composition of blubber collected in 1998 from 170 common minke whales (*Balaenoptera acutorostrata*) was used to study population structure in the North Atlantic. Samples from seven IWC management units were analysed: West Greenland ('WG',  $n=69$ ); East Greenland ('CG',  $n=3$ ); Jan Mayen ('CM',  $n=24$ ); Svalbard ('ES',  $n=16$ ); the Barents Sea ('EB',  $n=30$ ); Vestfjorden/Lofoten ('EC',  $n=7$ ); and the North Sea ('EN',  $n=21$ ). FA analyses were conducted on both deep and superficial blubber with a one-step extraction and esterification method followed by gas-chromatography. The 43 FAs identified comprised 93–99% of total FAs. CART and MANOVA analyses on FA signatures in both blubber sections suggested a '3-geographic Regions model' where the regions were Greenland (WG, CG), the Northeast Atlantic (CM, ES, EB, EC) and the North Sea (EN). This is in general agreement with a genetic study on the same samples and suggests that differences in FA signatures can be used for studying population structure in minke whales. Potential variation in FA signatures caused by internal and environmental factors needs to be better understood. It is recommended that future studies of blubber FA signatures in minke whales include samples from their entire North Atlantic range (including Canadian and Icelandic waters). Samples should be collected from a pre-specified body site to rule out possible internal variation and during a narrow time-window in the same year to rule out seasonal exchange between areas.

KEYWORDS: COMMON MINKE WHALE; STOCK STRUCTURE; POPULATION; FATTY ACIDS; GREENLAND; NORTH ATLANTIC; NORTH SEA

## INTRODUCTION

The common minke whale (*Balaenoptera acutorostrata*) is the smallest and most abundant of the baleen whales in the North Atlantic (e.g. Stewart and Leatherwood, 1985; Donovan, 1991a; b). During summer, minke whales are distributed from the east coast of Canada to the North Sea, and as far northeast as the Svalbard-Barents Sea region (Fig. 1). This species is exploited by Greenlandic subsistence hunters in coastal Greenland waters (IWC, 2003, pp.68–70) and by Norwegian whalers along the Norwegian coast and offshore in the North East Atlantic region and the North Sea (e.g. Grønvik, 1998). Determining sustainable harvest levels for minke whales in these areas requires an understanding of the population structure and the ability to identify the exploited units demographically.

In 1977, the International Whaling Commission (IWC) divided the North Atlantic minke whale population into four management stocks: (1) West Greenland; (2) Central North Atlantic; (3) NE North Atlantic; and (4) Canadian East Coast (Donovan, 1991b). However, the evidence for some of these was 'somewhat scanty' and there have been a number of suggestions for changes and improvements (e.g. Larsen and Øien, 1988; Bakke *et al.*, 1996; Palsboll *et al.*, 1997). With the development of the Revised Management Procedure, the Committee re-evaluated the evidence and divided the North

Atlantic into 10 'Small Areas'<sup>1</sup> (IWC, 1993; 1994) (Fig. 1).

In the North Atlantic and elsewhere, genetic data have proved equivocal information on stock structure (e.g. IWC, 2004) and it is important that information from a variety of techniques is examined (e.g. Donovan, 1991b). Other studies have applied various techniques including comparison of catch composition (e.g. Larsen and Øien, 1988), morphological differences (Christensen *et al.*, 1990) and reproductive parameters (Olsen, 1997), but have not provided a definite answer to this question. However, new analytical tools that reflect changes over a shorter time-scale compared to genetics may assist in the understanding of the population structure of North Atlantic minke whales. One such tool is the composition of fatty acids (FAs) in depot fats such as the blubber of marine mammals. Examples where FAs have been used as a tool to discriminate between

<sup>1</sup>The formal definition is that 'Small Areas are disjoint areas small enough to contain whales from only one biological stock, or be such that if whales from different biological stocks are present in the Small Area, catching operations would not be able to harvest them in proportions substantially different to their proportions in the Small Area'. They are thus management units and do not have to have boundaries that coincide with biological stocks. Medium Areas 'correspond to known or suspected ranges of distinct biological stocks'. (IWC, 1999).

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populations include: ringed seals, *Phoca hispida* (Käkelä *et al.*, 1993); harp seals, *Phoca groenlandica* (Grahnl-Nielsen *et al.*, 1993); harbour seals, *Phoca vitulina* (Smith *et al.*, 1996; Iverson *et al.*, 1997); and harbour porpoises, *Phocoena phocoena* (Møller *et al.*, 2003). In addition, Olsen and Grahnl-Nielsen (2002) were able to differentiate between minke whales from the Norwegian Sea and the North Sea using differences in FA signatures in blubber.

In marine mammals the dietary FAs are represented mainly by long chain mono-unsaturated (e.g. C18:1n-7/n-9, C20:1n-9/n-11, C22:1n-9/n-11) and poly-unsaturated fatty acids (e.g. C18:3n-3, 18:4n-3, 20:4n-6, C20:5n-3, C22:5n-3, 22:6n-3) (e.g. Ackman *et al.*, 1975; West *et al.*, 1979; Koopman *et al.*, 1996; Iverson *et al.*, 1997; Smith *et al.*, 1997; Walton *et al.*, 2000).

Previous genetic studies compared minke whales collected in different years, making it difficult to distinguish between spatial and potential temporal differentiation (IWC, 1998). To eliminate some of these uncertainties, this study used minke whales caught during a single whaling season in seven of 10 IWC 'Small Areas' in the North Atlantic. To date, the samples have been analysed for regional differences in signatures of elements and stable isotopes (Born *et al.*, 2003), organochlorines (Hobbs *et al.*, 2002), genetics (Andersen *et al.*, 2003) and caesium-137 (Born *et al.*, 2002).

This paper provides an analysis of the fatty acids in the deep and superficial blubber from 170 minke whales sampled in 1998 in West Greenland, the northeastern Atlantic and the North Sea with the objective of elucidating population structure. Information is presented on the FAs identified, and on the regional variation in the composition of the FAs in minke whales. Preliminary analyses were presented by Møller *et al.* (2000).

## MATERIALS AND METHODS

### Field sampling

Blubber samples were collected from 6 May until 31 October 1998 from 170 minke whales taken during directed catches by Greenlandic and Norwegian whalers in the North Atlantic region (Fig. 1): West Greenland ('WG',  $n=37$ ); East Greenland ('CG'  $n=3$ ); Jan Mayen ('CM',  $n=24$ ); Svalbard ('ES',  $n=16$ ); the Barents Sea ('EB',  $n=30$ ); Vestfjorden/Lofoten ('EC',  $n=7$ ); and the North Sea ('EN',  $n=21$ ). Within the same period additional samples were collected in Greenland from 32 minke whales. These samples, for which exact information on site and date was not available, were grouped together with the three animals from CG to form a mixed CG and WG group, from here on referred to as 'GR' ( $n=35$ ).

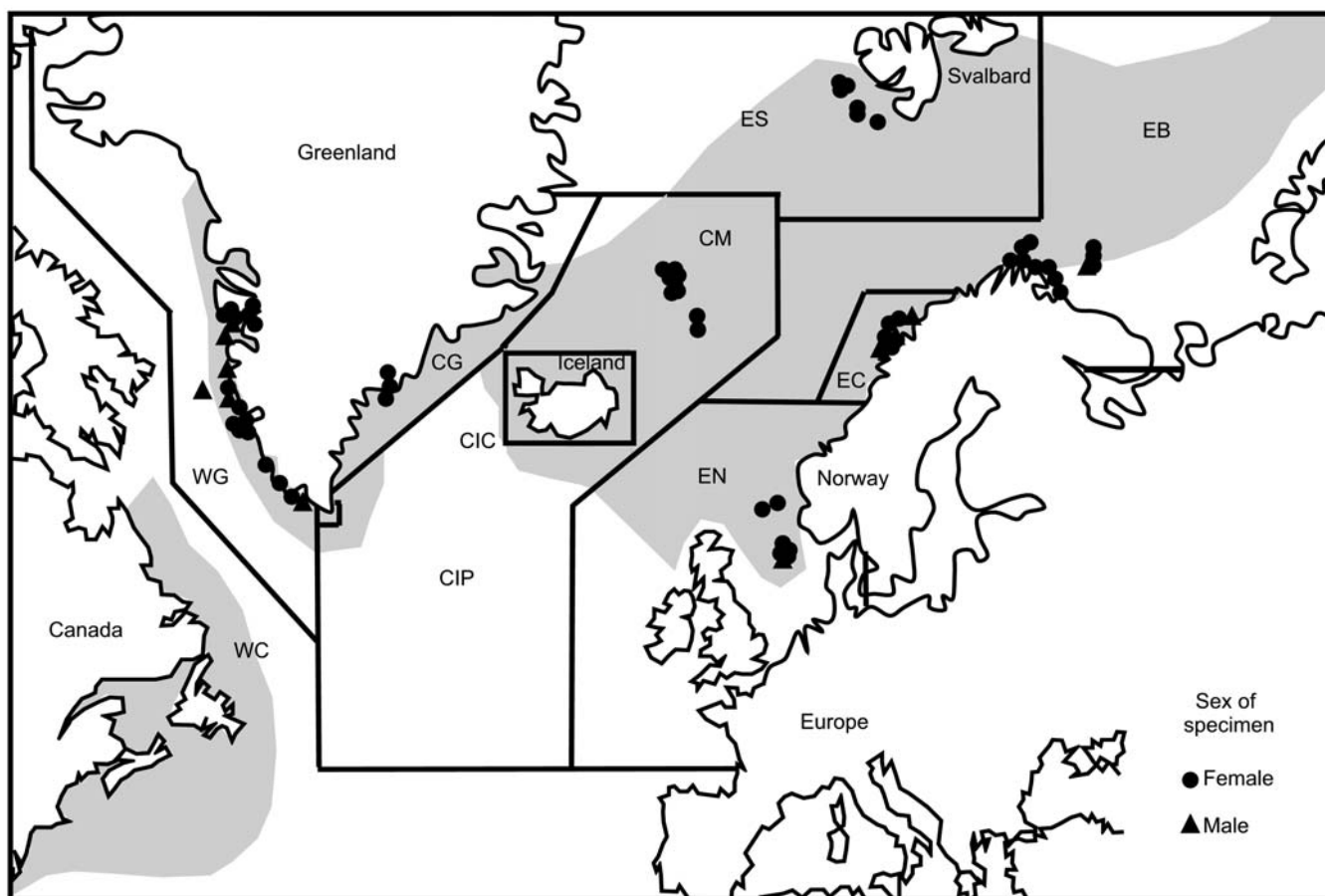


Fig. 1. Map showing the boundaries of the IWC 'Small Areas' and the location of sampling of tissues from a total of 170 minke whales in 1998. The approximate summer range of minke whales (Stewart and Leatherwood, 1985; Donovan, 1991a; b) is indicated in grey. The areas west of 57°W (i.e. the central parts of Davis Strait and the Canadian East Coast waters) have not been surveyed systematically and therefore it is not known whether or not the distribution of minke whales is continuous between western Greenland and Canada.] Key = WC (West Canada); WG (West Greenland); CG (Central Greenland); CIC (Central Island Coastal); CIP (Central Iceland Pelagic); CM (Central Jan Mayen); ES (East Svalbard); EB (East Barents Sea); EC (East Coastal); EN (East North Sea).

Samples for analyses were taken only if sub-samples included skin or muscle for correct orientation. This selection procedure resulted in deep blubber samples from 154 animals and superficial blubber samples from 164 animals representing 170 animals. Both the deep and superficial blubber were sampled from 148 minke whales.

A deep blubber core including skin and muscle was collected from each whale and stored at  $-20^{\circ}\text{C}$ . The sex of each individual was determined genetically (Andersen *et al.*, 2003). The overall percentage of females in the samples was 79% ranging between 50% (EC) and 100% (CG).

### Sample preparation and fatty acid methyl-esters

In September 1999, sub-samples representing the centre core of an entire blubber profile were transferred to polyethylene plastic bags where air was evacuated and samples stored at  $-80^{\circ}\text{C}$  until analysis. For analysis, sub-samples were thawed and placed on oil-free paper where a 2–3mm thick blubber layer was dissected from (a) immediately under the skin, and (b) adjacent to the muscle core.

Following this procedure, the individual layers were transferred to thick-walled glass tubes to be sealed with screw-caps fitted with a silicone-PTFE cap-membrane. Lipids were extracted and FAs trans-esterified to produce FA methyl-esters (FAME) using a one-step method (Sukhija and Palmquist, 1988) as modified by Møller (1999). FAMES were stored in air-sealed GC-vials at  $-80^{\circ}\text{C}$  until the identification-analysis could be performed (0–5 days). To avoid auto-oxidation of unsaturated FAs, all chemicals and headspace volumes were de-aerated using purified argon gas.

To avoid loss of particular volatile short-chained FAs (e.g. isovaleric acid) the use of FA butyl-esters (FABE) instead of the commonly used FAME has been recommended. However, analyses on blubber FABE in minke whales have shown no presence of such volatile FAs (P.M., unpublished data) and for convenience FAME (referred to as FAs in the following) were therefore chosen for this study.

### FA analysis

The FAs were analysed and identified using a *Hewlett Packard* 5890 gas-chromatograph equipped with a split/splitless FID detector. A  $30 \times 0.25\text{mm}$  internal diameter column coated with 50% cyanopropyl polysiloxane (0.247mm film thickness; J&W DB-23; Folsom California) was used. Helium was used as the inert carrier gas at a constant flow of 1.2ml/min. Injection- and detection-temperatures were set at  $250^{\circ}\text{C}$  and the initial column temperature at  $65^{\circ}\text{C}$ . Two minutes after sample injection the temperature was increased from  $65^{\circ}\text{C}$  to  $165^{\circ}\text{C}$  at  $20^{\circ}\text{C}/\text{min}$  and held for 0.4min. The temperature was then increased to  $210^{\circ}\text{C}$  at  $2^{\circ}\text{C}/\text{min}$ , held for 1min, and then finally increased to  $240^{\circ}\text{C}$  at  $30^{\circ}\text{C}/\text{min}$  and held for 1min. The entire program took 32.9min to complete. The Hewlett Packard ChemStation software (HP 3363 Series II ChemStation) performed integration of chromatograms. Identification of most individual FAs was performed using methyl-ester standard mixes FIM-FAME-7 and PUFA-3 (Matreya, Inc.). FAs of the n-11 and n-9 type were identified using an oil-extract from harbour porpoise blubber of known composition. The integrated area peaks were converted to FA percentage by weight (mass percentage of total FAs) using theoretical correction factors (Craske and Bannon, 1988; Møller, 1999). Standards were run before and after sample-sequences to calibrate the retention times and to monitor the condition of the column. Individual FAs have been named according to the short-hand IUPAC

nomenclature: C(#carbon):(double bonds)n-x, where x is the location of the double bond nearest the terminal methyl group.

### Data analysis

Classification and Regression Tree analysis (CART), ANOVA and MANOVA available in *S-plus*® (version 4.5, Mathsoft, Inc.) were used to investigate patterns in the FA signatures among: (a) IWC 'Small Areas'; and (b) major regions i.e. Greenland (CG, WG), the NE Atlantic region (CM, ES, EB and EC pooled) and the North Sea (EN) (Fig. 1). In contrast to ANOVA and MANOVA, CART multivariate analysis (Clark and Pregibon, 1992; Venables and Ripley, 1994) is non-parametric and has no restriction as to the number of variables allowed in the model. Therefore the total array of FAs was tested when using CART. The CART technique has previously been applied to the analysis of FA signatures containing more than 60 variables (FAs) per observation (Iverson *et al.*, 1997; Smith *et al.*, 1997). An initial CART analysis revealed similar patterns for males and females and the two genders were therefore pooled in subsequent analyses of spatial differences. The deep blubber and the superficial blubber were analysed separately. Prior to analysis, the data were arcsin transformed to meet the assumption of normality and homoscedasticity. For construction of the classification trees, two stopping criteria were used to determine branches: (1) a change in deviation of less than 1% of the root node deviation; or (2) when the minimum number of observations at a node was less than 10.

A '3-Region model' and a '2-Region model' as suggested by the CART analysis was tested further by use of multivariable analyses of variance (MANOVA; Wilks  $\lambda$ ) including a total of 18 FAs. Furthermore, analyses of variance (ANOVA) were conducted to indicate the probable importance of individual FAs included in the MANOVA. These 18 FAs were those responsible for the major splits picked up by the CART analyses and other FAs of dietary origin.

## RESULTS

The 43 FAs identified in this study made up 93–99% of total FAs in the blubber of the minke whales. Of these FAs, the following 16 were generally represented by  $> 1\%$  on weight basis: 14:0, 16:0, 16:1n-7, 18:0, 18:1n-11, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 18:4n-3, 20:1n-11, 20:1n-9, 20:5n-3, 22:1n-11, 22:5n-3, 22:6n-3 (Table 1).

### Regional differences based on CART analyses

#### Deep blubber signatures

Based on the FAs in the deep blubber, the overall percentage of misclassification of individuals to area was 17% (i.e. 26 misclassified of 154 analysed, 26/154). The model selected 19 of 43 FAs for the construction of a classification tree with 19 terminal nodes (Fig. 2). At the root C20:1n-7 formed an initial split into a NE Atlantic-North Sea group (3/69, 3 misclassified of 69 classified) and a NE Atlantic-Greenland group (0/85). Only 3 out of 64 Greenland animals were misclassified to the NE Atlantic-North Sea group. In addition, all 20 North Sea animals were found in this group where all but one could successfully be categorised in a clean terminal node (0/19). Within the NE Atlantic-Greenland group C18:4n-1 distinguished between NE Atlantic (9/32) and Greenland animals (1/53). In the Greenland group the one misclassified animal was from the neighbouring Jan Mayen area (CM). In both NE Atlantic sub-groups, Jan



Table 1

Fatty acid composition (average mass (%)  $\pm$  standard deviation) of the deep and the superficial blubber of minke whales representing the three regions, Greenland, the North Eastern Atlantic and the North Sea.

Fatty acid	Greenland		North Eastern Atlantic		North Sea	
	Deep	Superficial	Deep	Superficial	Deep	Superficial
C10:0	0.01 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.01	0.00 $\pm$ 0.00	0.02 $\pm$ 0.01	0.00 $\pm$ 0.00
C12:0	0.14 $\pm$ 0.05	0.09 $\pm$ 0.03	0.08 $\pm$ 0.03	0.07 $\pm$ 0.01	0.12 $\pm$ 0.02	0.07 $\pm$ 0.01
C13:0	0.03 $\pm$ 0.01	0.01 $\pm$ 0.02	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.01 $\pm$ 0.02
C14:0	4.80 $\pm$ 0.56	4.21 $\pm$ 0.45	5.54 $\pm$ 0.74	4.15 $\pm$ 0.37	4.99 $\pm$ 0.54	4.37 $\pm$ 0.51
C14:1n-7	0.22 $\pm$ 0.10	0.08 $\pm$ 0.02	0.22 $\pm$ 0.15	0.06 $\pm$ 0.02	0.14 $\pm$ 0.05	0.09 $\pm$ 0.04
C14:1n-5	0.22 $\pm$ 0.05	0.72 $\pm$ 0.30	0.17 $\pm$ 0.05	0.92 $\pm$ 0.19	0.17 $\pm$ 0.01	0.99 $\pm$ 0.38
C15:0	0.27 $\pm$ 0.08	0.29 $\pm$ 0.05	0.32 $\pm$ 0.06	0.31 $\pm$ 0.08	0.30 $\pm$ 0.05	0.28 $\pm$ 0.10
C15:1n-5	0.06 $\pm$ 0.05	0.09 $\pm$ 0.04	0.02 $\pm$ 0.07	0.09 $\pm$ 0.03	0.11 $\pm$ 0.06	0.09 $\pm$ 0.03
C16:0	10.73 $\pm$ 2.18	8.71 $\pm$ 2.18	14.46 $\pm$ 2.45	8.49 $\pm$ 1.34	10.32 $\pm$ 2.12	7.28 $\pm$ 1.34
C16:1n-7	7.78 $\pm$ 2.46	13.45 $\pm$ 2.63	6.88 $\pm$ 2.51	13.06 $\pm$ 2.21	5.61 $\pm$ 2.33	10.66 $\pm$ 1.79
C16:2n-4	0.44 $\pm$ 0.13	0.54 $\pm$ 0.10	0.38 $\pm$ 0.11	0.36 $\pm$ 0.10	0.34 $\pm$ 0.09	0.35 $\pm$ 0.08
C17:0	0.42 $\pm$ 0.25	0.27 $\pm$ 0.08	0.51 $\pm$ 0.31	0.20 $\pm$ 0.12	0.31 $\pm$ 0.12	0.25 $\pm$ 0.11
C16:3n-4	0.29 $\pm$ 0.15	0.34 $\pm$ 0.12	0.33 $\pm$ 0.10	0.48 $\pm$ 0.13	0.39 $\pm$ 0.08	0.47 $\pm$ 0.14
C17:1n-7	0.04 $\pm$ 0.03	0.20 $\pm$ 0.10	0.10 $\pm$ 0.06	0.12 $\pm$ 0.11	0.05 $\pm$ 0.04	0.17 $\pm$ 0.10
C16:4n-1	0.42 $\pm$ 0.25	0.18 $\pm$ 0.11	0.18 $\pm$ 0.11	0.10 $\pm$ 0.20	0.39 $\pm$ 0.15	0.14 $\pm$ 0.15
C18:0	2.45 $\pm$ 0.68	1.84 $\pm$ 0.44	2.54 $\pm$ 0.77	1.77 $\pm$ 0.33	2.75 $\pm$ 0.59	1.84 $\pm$ 0.46
C18:1n-11	1.54 $\pm$ 0.74	2.60 $\pm$ 0.94	0.82 $\pm$ 0.64	2.15 $\pm$ 0.61	1.61 $\pm$ 0.63	2.70 $\pm$ 0.83
C18:1n-9	13.80 $\pm$ 3.44	18.31 $\pm$ 2.84	21.35 $\pm$ 4.44	22.16 $\pm$ 2.23	11.22 $\pm$ 2.73	18.63 $\pm$ 2.03
C18:1n-7	3.71 $\pm$ 1.14	4.52 $\pm$ 0.95	5.19 $\pm$ 1.76	4.86 $\pm$ 0.99	1.84 $\pm$ 0.23	2.93 $\pm$ 0.63
C18:2n-6	1.33 $\pm$ 0.28	1.64 $\pm$ 0.25	1.28 $\pm$ 0.35	1.93 $\pm$ 0.20	2.16 $\pm$ 0.43	2.34 $\pm$ 0.35
C18:2n-4	0.12 $\pm$ 0.04	0.09 $\pm$ 0.05	0.07 $\pm$ 0.04	0.08 $\pm$ 0.05	0.09 $\pm$ 0.04	0.05 $\pm$ 0.05
C18:3n-6	0.10 $\pm$ 0.03	0.02 $\pm$ 0.05	0.12 $\pm$ 0.04	0.10 $\pm$ 0.09	0.10 $\pm$ 0.07	0.06 $\pm$ 0.07
C18:3n-4	0.14 $\pm$ 0.04	0.65 $\pm$ 0.16	0.10 $\pm$ 0.05	0.95 $\pm$ 0.21	0.12 $\pm$ 0.03	1.40 $\pm$ 0.24
C18:3n-3	0.52 $\pm$ 0.14	0.95 $\pm$ 0.38	0.66 $\pm$ 0.21	1.03 $\pm$ 0.20	1.11 $\pm$ 0.39	1.47 $\pm$ 0.28
C18:4n-3	1.60 $\pm$ 0.58	0.33 $\pm$ 0.13	1.81 $\pm$ 0.67	0.22 $\pm$ 0.18	2.32 $\pm$ 0.69	0.18 $\pm$ 0.07
C18:4n-1	0.24 $\pm$ 0.11	0.00 $\pm$ 0.00	0.10 $\pm$ 0.06	0.01 $\pm$ 0.03	0.20 $\pm$ 0.07	0.00 $\pm$ 0.00
C20:0	0.16 $\pm$ 0.06	0.08 $\pm$ 0.08	0.18 $\pm$ 0.08	0.11 $\pm$ 0.06	0.24 $\pm$ 0.06	0.11 $\pm$ 0.07
C20:1n-11	1.80 $\pm$ 1.19	2.62 $\pm$ 0.88	1.14 $\pm$ 1.00	2.32 $\pm$ 0.70	2.37 $\pm$ 1.07	3.03 $\pm$ 0.81
C20:1n-9	11.42 $\pm$ 3.84	10.91 $\pm$ 3.30	7.45 $\pm$ 4.19	9.26 $\pm$ 2.84	10.97 $\pm$ 3.14	10.44 $\pm$ 2.57
C20:1n-7	0.73 $\pm$ 0.93	0.50 $\pm$ 0.15	0.33 $\pm$ 0.12	0.35 $\pm$ 0.06	0.23 $\pm$ 0.05	0.25 $\pm$ 0.04
C20:2n-6	0.33 $\pm$ 0.09	0.30 $\pm$ 0.07	0.34 $\pm$ 0.10	0.32 $\pm$ 0.05	0.36 $\pm$ 0.05	0.39 $\pm$ 0.05
C20:3n-6	0.11 $\pm$ 0.10	0.05 $\pm$ 0.07	0.10 $\pm$ 0.04	0.08 $\pm$ 0.06	0.10 $\pm$ 0.05	0.06 $\pm$ 0.05
C20:4n-6	0.29 $\pm$ 0.11	0.36 $\pm$ 0.10	0.30 $\pm$ 0.10	0.37 $\pm$ 0.10	0.40 $\pm$ 0.12	0.42 $\pm$ 0.10
C20:3n-3	0.07 $\pm$ 0.02	0.01 $\pm$ 0.03	0.09 $\pm$ 0.04	0.07 $\pm$ 0.05	0.10 $\pm$ 0.05	0.10 $\pm$ 0.08
C20:4n-3	0.89 $\pm$ 0.21	0.98 $\pm$ 0.26	0.84 $\pm$ 0.21	1.19 $\pm$ 0.22	1.12 $\pm$ 0.26	1.37 $\pm$ 0.27
C20:5n-3	6.29 $\pm$ 2.85	4.59 $\pm$ 1.60	5.50 $\pm$ 1.47	4.00 $\pm$ 1.24	5.61 $\pm$ 2.26	3.45 $\pm$ 1.20
C22:0	0.04 $\pm$ 0.02	0.00 $\pm$ 0.01	0.02 $\pm$ 0.03	0.01 $\pm$ 0.01	0.02 $\pm$ 0.03	0.01 $\pm$ 0.01
C22:1n-11	9.08 $\pm$ 3.47	7.37 $\pm$ 3.04	7.30 $\pm$ 4.39	6.20 $\pm$ 2.43	12.72 $\pm$ 3.64	10.18 $\pm$ 3.47
C22:1n-9	1.01 $\pm$ 0.35	0.90 $\pm$ 1.00	0.56 $\pm$ 0.37	0.58 $\pm$ 0.16	0.83 $\pm$ 0.19	0.60 $\pm$ 0.12
C22:2n-6	0.40 $\pm$ 0.16	0.24 $\pm$ 0.10	0.40 $\pm$ 0.17	0.26 $\pm$ 0.08	0.54 $\pm$ 0.14	0.27 $\pm$ 0.11
C22:5n-3	2.82 $\pm$ 0.65	2.64 $\pm$ 0.62	2.30 $\pm$ 0.65	2.55 $\pm$ 0.61	2.68 $\pm$ 0.46	2.38 $\pm$ 0.74
C22:6n-3	6.75 $\pm$ 1.59	5.01 $\pm$ 2.00	5.37 $\pm$ 2.03	4.79 $\pm$ 1.88	8.06 $\pm$ 2.45	5.79 $\pm$ 2.25
C24:1n-9	0.41 $\pm$ 0.18	0.19 $\pm$ 0.10	0.31 $\pm$ 0.21	0.17 $\pm$ 0.08	0.79 $\pm$ 0.15	0.20 $\pm$ 0.12
Ident. FA	93.97 $\pm$ 0.95	96.87 $\pm$ 0.60	95.80 $\pm$ 1.15	96.34 $\pm$ 0.97	93.94 $\pm$ 1.06	95.90 $\pm$ 1.10
Not ident. FA	6.03 $\pm$ 0.95	3.13 $\pm$ 0.60	4.20 $\pm$ 1.15	3.66 $\pm$ 0.97	6.06 $\pm$ 1.06	4.10 $\pm$ 1.10
Sample size	64	71	70	74	20	19

Mayen and Greenland animals (GR and WG) intermingled while a lumping of Vestfjorden/Lofoten (EC) and Svalbard (ES) was apparent. The whales from the Barents Sea (EB) showed a more pronounced isolation from the rest of the NE Atlantic (i.e. 0/16 and 1/7). Approximately half of the Jan Mayen animals were isolated from the rest of the NE Atlantic and lumped together with the North Sea animals.

#### Superficial blubber signatures

The CART analysis based on FAs in the superficial blubber resulted in a slightly higher rate of misclassifications (22.0%, 36 misclassified of 164) (Fig. 3) than found for the deep blubber. The model selected 19 FAs to produce a total of 20 terminal nodes. At the root node, C18:3n-4 distinguished between animals from Greenland and the NE

Atlantic-North Sea with a misclassification rate of 12.2% (20/164). All of the 19 North Sea animals were along the NE Atlantic-North Sea branch where 17 were categorised correctly into a terminal North Sea (EN) node. The only misclassification at this node represented an animal from the neighbouring Vestfjorden/Lofoten (EC) area. Similarities between Jan Mayen (CM) and Greenland animals were observed as indicated by a general intermingling between animals from these two areas (Fig. 3).

#### Conclusion

The FA signatures of both the deep and the superficial blubber indicated: (1) that North Sea minke whales differed from those sampled in the northeastern Atlantic (i.e. Jan Mayen, Svalbard, Barents Sea and Vestfjorden/Lofoten) and

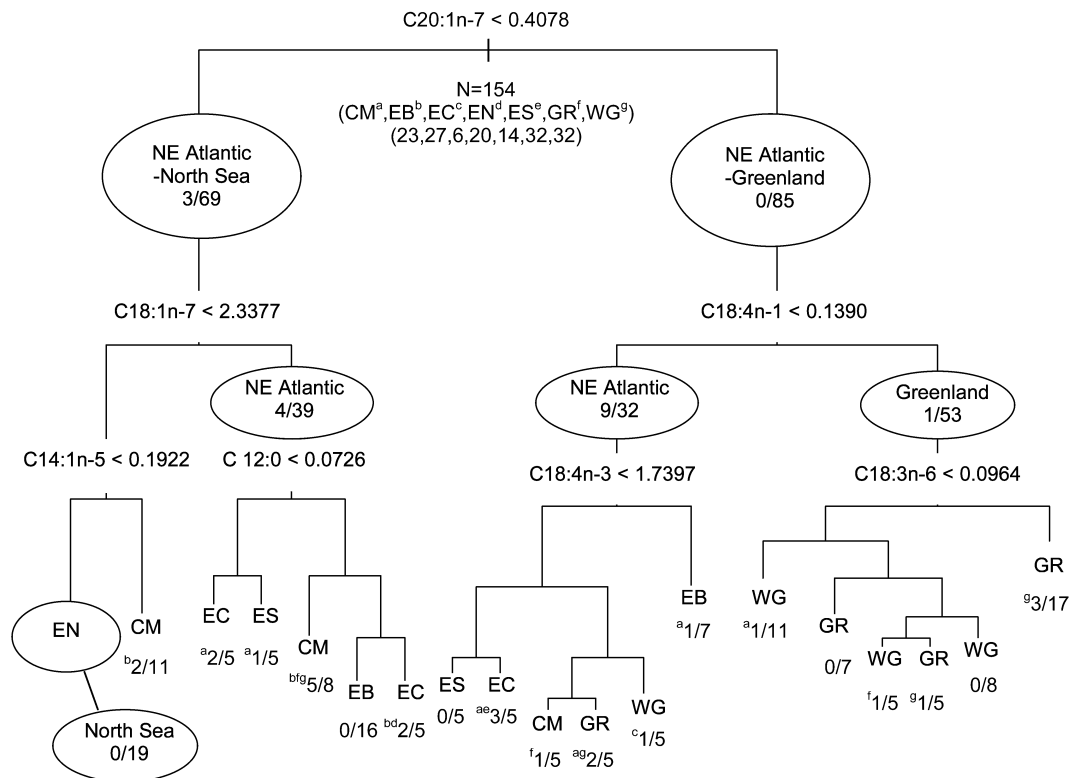


Fig. 2. Classification of 154 minke whales according to IWC 'Small Areas' in the North Atlantic using CART analyses on fatty acid (FA) signatures of the deep blubber. Overall misclassification rate = 17% (26/154). Fractions represent the number of misclassified individuals over the total number of individuals classified in a given category. Letters in superscript refer to the 'origin' of misclassified individuals where individual codes (i.e. a to g) are indicated at the root node. Only FAs responsible for the major branches have been included in the figure.

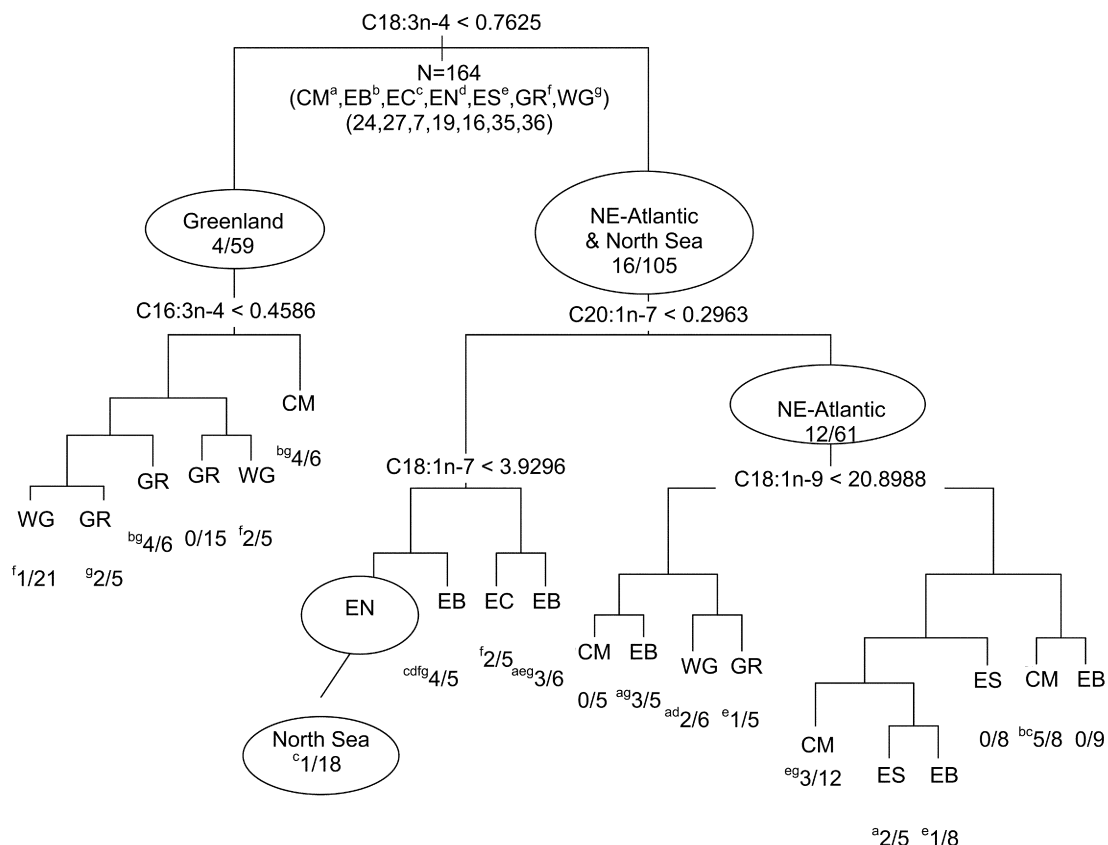


Fig. 3. Classification of 164 minke whales according to IWC 'Small Areas' in the North Atlantic using CART analyses on fatty acid (FA) signatures of the superficial blubber. Overall misclassification rate = 22% (36/164). Fractions represent the number of misclassified individuals over the total number of individuals classified in a given category. Letters in superscript refer to the 'origin' of misclassified individuals where individual codes (i.e. a to g) are indicated at the root node. Only FAs responsible for the major branches have been included in the figure.

Greenland; and (2) that animals from Greenland waters differed from NE Atlantic minke whales; (3) a similarity between CM and Greenland; and (4) minke whales from the Barents Sea appeared to be somewhat different from the rest of the NE Atlantic. Although the FAs responsible for the tree construction differed between blubber layers, the complexity and overall topography of the trees did not.

### Regional differences based on MANOVA and ANOVA

Eighteen principal FAs were included in the analyses of regional differences using MANOVA (Fig. 4). The FA composition of both the deep and the superficial blubber layers differed significantly among (a) IWC 'Small Areas'; and (b) among three regions in a '3-Region model' (i.e. all-Greenland versus NE Atlantic versus Eastern North Sea) ( $p < 0.0001$ , Table 2). However, the largest  $F$ -value resulted from the analysis of the '3-Region model'. Furthermore, a MANOVA on Eastern North Sea versus NE Atlantic supported the 3-Region model ( $p < 0.0001$ ) (Table 2). The ANOVAs performed on the 18 individual FAs that were included in the MANOVA test of the '3-Region model' showed that six FAs in the deep blubber and seven in the superficial blubber were responsible for the significant differences in FA signatures among areas (Fig. 4). In three cases, the same FAs found both in the outer and inner blubber were involved in these differences.

## DISCUSSION

### Location of the tissue samples

In no instances, except for the North Sea, were the same FAs picked up by the tree functions from both the deep and the superficial blubber layer. This emphasises that the two layers likely represent different metabolic histories. The blubber layer of the North Atlantic minke whale is stratified in such a way that the FA composition in the superficial layer differs from that in the deep blubber (Fehn, 1996; Møller *et al.*, 2000; Olsen and Grahl-Nielsen, 2002). A similar stratification has been described for several other marine mammals (West *et al.*, 1979; Lockyer *et al.*, 1984; Fredheim *et al.*, 1995; Kåkelä and Hyvärinen, 1996; Koopman *et al.*, 1996; Møller *et al.*, 2002). These studies suggest that the superficial blubber layer is a region for storage of relatively endogenous FAs with its main function being insulation. In contrast, the deep blubber layer has a higher degree of unsaturation and is thought to be metabolically more active.

The attempts to distinguish among all IWC 'Small Areas' resulted in relatively high percentages of misclassification both for the deep and the superficial blubber layer (17% and 22%, respectively). However, included in these percentages are misclassified animals from the mixed Greenland group (GR) representing 3 East Greenland animals and 32 Greenland animals with no exact information on sampling area (i.e. CG or WG). Animals from this group could in fact represent 'false' misclassifications. Consequently, a clear distinction between samples from the different IWC 'Small Areas' was not possible, although given that 'Small Areas' are not intended to correspond to separate biological stocks, this is not surprising. However, the classification trees constructed on the deep and the superficial blubber both indicated that whales sampled in Greenland differed from those from the NE Atlantic-North Sea region (Figs 2 and 3). In addition, CART analyses indicated that minke whales from the North Sea area (EN) differed from the NE-Atlantic minke whales. The MANOVA supported the existence of both a '2-Region model' (Greenland versus NE

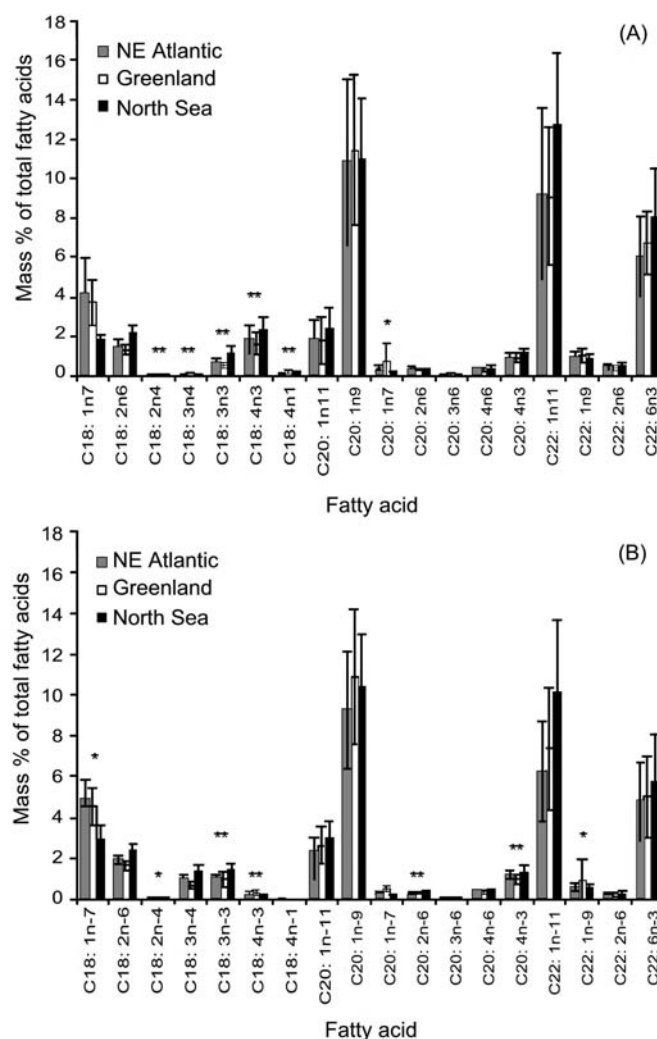


Fig. 4. Results of ANOVAs performed on 18 fatty acids in blubber of minke whales that were sampled in the North Atlantic region during 1998. Bars and lines represent the mean and standard deviation, respectively, by major region (NE Atlantic, Greenland, North Sea) as identified from the CART analyses. (A) deep blubber; (B) superficial blubber (\* =  $p < 0.01$ , \*\* =  $p < 0.001$ ).

Table 2

MANOVA analysis for the effect of areas on the concentration of 18 fatty acids in the blubber of minke whales from the North Atlantic (1998).

(A) = deep blubber and (B) = superficial blubber.

Variable	Df	Pillai	Approx F	Num df	Denom df	P-value
<b>(A)</b>						
IWC small areas*	1	0.534	8.589	18	135	< 0.0001
3 areas**	1	0.731	20.373	18	135	< 0.0001
Residuals	152					
Eastern North Sea vs NE Atlantic	1	0.702	9.27	18	71	< 0.0001
Residuals	88					
<b>(B)</b>						
IWC small areas*	1	0.418	5.716	18	143	< 0.0001
3 areas**	1	0.699	18.438	18	143	< 0.0001
Residuals	160					
Eastern North Sea vs NE Atlantic	1	0.683	8.731	18	73	< 0.0001
Residuals	90					

\*Six groups representing IWC small areas and one which represents animals from two IWC small areas (i.e. West and East Greenland).

\*\*Greenland vs North East Atlantic vs Eastern North Sea.

Atlantic-North Sea) and a '3-Region model' (Greenland vs. NE Atlantic vs the North Sea) model. However, larger *F*-values were obtained from testing the '3-Region model' for both blubber layers thereby favouring this model over the '2-Region model'. This effectively is in accord with IWC 'Medium Area' assumptions of three biological stocks (IWC, 2004). The MANOVA also indicated that there were significant differences among some IWC 'Small Areas'. However, because a total of 43 FAs were included in the construction of classification trees, the CART analyses are thought to be relatively more powerful than the MANOVA in separating among whales sampled in different areas. Differences in FA signatures in this study may have been influenced by the fact that samples may have been taken from different parts of the body of the whale. Differences in blubber FA composition between two dorsal sites (30cm in front of and 30cm behind the dorsal fin) have been reported for North Atlantic minke whales (Olsen and Grahl-Nielsen, 2002). However, this difference was much smaller than the difference in FA signatures between the deep and superficial layer (Olsen and Grahl-Nielsen, 2002). No information is available about the exact sites from where the samples were taken and therefore the potential influence of the uncertainty associated with the sampling method is difficult to assess. There is no indication that large (>7m) and small (<7m) whales feed on different food items (Haug *et al.*, 2002), but differences according to sexual maturity have been identified in harbour porpoise (Møller, 1999) and may also influence the results of this study to some degree. However, the fact that the findings in this study resemble those obtained in a genetic study using the same samples (Anderson *et al.*, 2002) indicates that the FA technique is useful irrespective of sexual status and the location of the blubber sample on the whale.

#### Animal movements

The lack of clear differences among regions could to some extent be explained by some animals moving rapidly among feeding grounds. Minke whales are capable of relatively high swimming speeds (i.e. 7-12km/h, Blix and Folkow, 1995; Folkow, in litt., 27 April 2000). Therefore, a directed movement between even distant areas within the range of this study may take a minke whale only a few weeks. Hence, a whale may have fed in one area to be sampled not much later in another area. Furthermore, the actual lag-time between the dietary intake of the FAs and their deposition as a signal in the blubber is not known.

Despite the fact that the composition of FAs in the depot fats of marine mammals is influenced by the composition of the diet (e.g. Ackman, 1980), finding a FA composition in a predator identical to that of its diet is unusual (Iverson *et al.*, 1995). This can be explained by an animal's ability to *de novo* synthesise and selectively metabolise, absorb and deposit FAs (Enser, 1984; Sargeant *et al.*, 1988). It is a combination of dietary fats and endogenous synthesis that influences the blubber FA signature. Even though the diet of North Atlantic minke whales has been shown to vary considerably between geographic regions and periods (e.g. Haug *et al.*, 2002; Sigurjónsson and Galan, 1990; Lydersen *et al.*, 1991), it is a combination of internal and environmental factors that influences the composition of the blubber.

#### Additional sampling areas

Ideally, samples of minke whales from neighbouring Canadian (WC) and Icelandic (CIP and CIC, cf. Fig. 1) waters should have been included in this study. However,

minke whales are currently not harvested by Canada or Iceland. Further work on differences in FA signatures to incorporate minke whales from the entire North Atlantic range of this species is recommended. Samples from areas where minke whales are not harvested may in the future be obtained from biopsies taken from free-roaming whales. Knowledge of the metabolism of the blubber, the turnover rate of FAs, and the effect of e.g. physiological state and reproductive status of the individual may significantly advance the feasible use of FA signatures as a tool in population studies.

#### The influence of foraging

Blubber FA signatures may reflect major and sometimes even minor differences in the diet (e.g. Iverson *et al.*, 1997; Møller *et al.*, 2003). Within the range covered by this study, there are major differences in food available to and consequently eaten by minke whales. Minke whales concentrate on traditional summer feeding grounds (Solvik, 1976; Harwood, 1990) where they feed in shallow shelf areas in association with highly productive frontal regimes (Mann and Lazier, 1991). In the Northern Hemisphere no single organism forms a predominant food supply in the minke whale diet. The complex oceanography and bathymetry of the North Atlantic (Mackintosh, 1965) can in part explain this heterogeneity. Minke whales differ markedly among the regions within the range of this study with respect to diet (Folkow *et al.*, 2000; Neve, 2000; Olsen and Holst, 2001; Haug *et al.*, 2002). Capelin (*Mallotus villosus*) is an important food for minke whales in West Greenland waters whereas polar cod (*Boreogadus saida*) seems to be of relatively greater importance in eastern Greenland (Neve, 2000). Generally, the minke whale food composition in Greenland waters resembles that reported for Icelandic nearshore waters where capelin and sand eel (*Ammodytes* sp.) made up ca 56% and krill (mainly *Thysanoessa* sp.) ca 35% of the food on weighted frequency basis (Sigurjónsson *et al.*, 2000).

Studies of minke whale diet in the Northeast Atlantic over the period 1992-1999 showed that the food comprised of relatively few species and that the dietary composition varied considerably both in space and time, presumably due to geographic differences in the distribution and abundance of potential prey (Haug *et al.*, 2002). In general, the whales find capelin and herring (*Clupea harengus*) and, occasionally, krill more preferably than other prey, which usually comprised of gadoid fish (cod, *Gadus morhua*; saithe, *Pollachius virens* and haddock, *Melanogrammus aeglefinus*). In the northeastern Atlantic, regional differences in stomach contents were found. Consumption of herring was almost exclusively confined to the Vestfjorden/Lofoten (EC) and the Barents Sea (EB) areas whereas consumption of krill was most pronounced in the Svalbard (ES) area (Folkow *et al.*, 2000). In the latter area, capelin was important prior to the collapse of the Barents Sea capelin stock in 1992-1993 (Haug *et al.*, 2002). In 1999, herring was a predominant food item in the Norwegian Sea whereas sand eel dominated (86.6% by weight) the minke whale food in the North Sea. In this latter area, mackerel (*Scomber scombrus*) made up 9.3% and other fish (e.g. herring) the remainder of food items (Olsen and Holst, 2001). These diets (stomach contents) are very different from those in Greenland waters where cod (*Gadus* sp.) has only been reported in a limited number of stomachs and herring in none (cf. Neve, 2000). The cod stock in Greenland during the 1990s has been very small (Anon., 2001) and herring, mackerel, saithe and haddock are almost absent (H.

Hovgård, Danish Fisheries Institute, DFU, pers. comm., 2001).

Although minke whales are euryphagous, and despite the fact that there are both inter-annual and inter-seasonal variation in their food, it is clear that their overall food selection is determined by prominent regional differences in the distribution and abundance of various prey types. Likely, these regional differences in prey availability are recorded as differences in signatures of FAs in the blubber of the minke whales. We believe that differences in the foraging ecology of minke whales among regions is recorded in the blubber layers. The deep layer likely provides a record of a more recent history in contrast to an older history recorded in the superficial blubber. However, there are no comparable data on regional differences in FAs in the fish species or in other prey of minke whales to allow for a thorough discussion on the trophic importance of the signatures found in the minke whales (e.g. Dietz *et al.*, 1998).

#### *Comparison with other studies*

Only one other study exists on regional differences in blubber FA signatures in North Atlantic baleen whales. Olsen (2002) used FAs to differentiate between minke whales sampled in the North Sea and the Norwegian Sea in 1999. The findings by Olsen (2002) supported the results of Møller *et al.* (2002) and this study, that minke whales from the North Sea constitute a group that is different from those summering further north in the NE Atlantic region.

The present study indicates the existence of population sub-structuring in North Atlantic minke whales on a large geographical scale. This is in accordance with other studies using the same material from 1998 but applying different analytical techniques. Genetic analyses, which included both mitochondrial and nuclear DNA suggested the existence of four genetically distinct subpopulations: (1) West Greenland; (2) East Greenland and Jan Mayen; (3) North East Atlantic (Svalbard, Barents Sea Vestfjorden/Lofoten); and (4) the North Sea (2002). Andersen *et al.* (2002) had access to a larger sample from East Greenland than the present study, which only included three samples from this region. This is the likely explanation for Andersen *et al.*'s (2002) finding that CG constitutes a separate unit.

A regional comparison of PCBs and organochlorine (OC) pesticides showed that minke whales from the Barents Sea (EB) had significantly higher concentrations of ΣPCBs than those from the Vestfjorden/Lofoten, the North Sea and Svalbard, as well as significantly higher ΣDDT concentrations compared to West Greenland animals (Hobbs *et al.*, 2002). The similarities and differences in concentrations suggested that minke whales from West Greenland and East Greenland represent one group of whales, distinct from both the Jan Mayen minke whales and those from other IWC defined stocks within the range covered by the present study. However, principal component analysis using proportions of OCs did not reveal any major differences among groups. With the exception of the Barents Sea and West Greenland, there was a general similarity in mean levels and proportions of OC contaminants among minke whales in the northeastern Atlantic suggesting that the minke whales are quite mobile and may feed in multiple areas.

Multivariate and principal component analyses of signatures of stable isotopes of Pb, C and N and 19 other elements in muscle, kidney, liver and baleen of the minke whales that were sampled in 1998 suggested the existence of sub-structuring of the minke whale population within the explored geographical range. In particular, minke whales in

West Greenland, the North Sea and the Vestfjorden/Lofoten areas appeared to be different from those in other areas (Born, *et al.*, 2003). Finally, Born *et al.* (2002) found the highest caesium-137 concentration in minke whales from the North Sea, and that the mean Cs-137 levels in minke whales from Svalbard and the North Sea differed significantly from mean levels in the other areas. This difference supports the indications from other studies that groups of minke whales are resident for some time at their feeding grounds in the North Atlantic and may occur in separate stocks during summer.

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# A multi-elemental approach to identification of sub-populations of North Atlantic minke whales (*Balaenoptera acutorostrata*)

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## Abstract

A combination of heavy metals, organochlorines (OC) and fatty acids (FA) that reflect long-term deposition (1+ year) in tissues were used in a Canonical Discriminant Analysis (CDA) exploring population sub-structure in 104 minke whales (*Balaenoptera acutorostrata*) that were sampled in West Greenland, the Central and Northeast Atlantic and the North Sea in 1998. A CDA that included mercury and cadmium in muscle, liver and kidney, and eight OCs and four unsaturated FAs in blubber was able to separate the whales into four sub-populations: (1) West Greenland, (2) a Central Atlantic group represented by whales from Jan Mayen, (3) a Northeast Atlantic group (Svalbard, Barents Sea and northwestern Norway), and (4) the North Sea. During an assignment test based on the data transformation developed by the CDA, about 84% of the individuals were correctly assigned to the area where they had been caught. The highest degree of mis-assignment was between Jan Mayen and the Northeast Atlantic group. The differences among the four groups likely reflected regional differences (i.e. sea water chemistry and prey type and prey availability) among the marine ecosystems within the range studied. The study indicated that a multi-elemental approach based on long-term deposited compounds with different ecological and physiological path-ways can be used for identification of sub-populations of marine mammals.

**Key words:** *Balaenoptera acutorostrata*, minke whale, multi-elemental analysis, North Atlantic, sub-population

## Introduction

Proper identification of sub-populations or “biological stocks” is a prerequisite for management that will ensure long-term sustainability of exploitation of wild animal species (e.g. Anon. 2002a). A biological population encompasses all the individuals in an area that are part of the same reproductive process. They form a self-contained unit, with emigration and immigration rates far lower than initial rate of population growth (ibid.). A sub-population may be defined as a geographically or otherwise distinct group in the population between which there is little exchange (Molloy et al. 2002).

Minke whales (*Balaenoptera acutorostrata*) range widely in the North Atlantic Ocean from the eastern coasts of the North American continent to Novaya Zemlya in the east (Fig. 1). The International Whaling Commission (IWC) divided North Atlantic minke whales into four major management areas (“stocks”) based mainly on segregation by sex and length, catch distribution, marking data and the distribution of the whales at their summer feeding grounds and considerations of ecological conditions. These four “stocks” were: Canadian East Coast, West Greenland, Central Atlantic (East Greenland-Iceland-Jan Mayen) and Northeastern Atlantic (Svalbard-Norway-British Isles) (Donovan 1991a). These areas have been further divided into 10 “management sub-areas” of “small areas” (Anon. 1994, 2004), Fig. 1.

Several studies involving analyses of genetics, morphometrics, and distributional and catch data have aimed at determining the population sub-structure of North Atlantic minke whales (reviewed in Anon. 1998, Waerebeek et al. 1999, Andersen et al. 2003, Anon. 2004). The studies indicated some sub-structuring but generally failed to find a clear distinction between minke whales from various regions of the North Atlantic. Most recent studies supported the hypothesis that minke whales from West Greenland and the North Sea differ from those in other sub-areas (Andersen et al. 2003, Møller et al. 2003, Born et al. 2002, 2003, Hobbs et al. 2003, Anon. 2004).

Four discrete groups of minke whales were identified genetically by Andersen et al. (2003) based on material sampled from West Greenland (1996, 1997, 1998, 1999) and across the NE Atlantic to the North Sea (1998). Using the suite of samples from 1998 the population sub-structure of minke whales was also studied using regional variation in muscle <sup>137</sup>Cs concentrations (Born et al. 2002), OC burdens (Hobbs et al. 2003), FA composition (Møller et al. 2003), and various elements including mercury (Hg) and cadmium (Cd) in soft tissues and baleen (Born et al. 2003).

The present study was made with the purpose to identify minke whale sub-populations that on a long-term scale have been geographically separated at those North Atlantic summering grounds where Greenland and Norway catch this species for human consumption (Grønvik 1998, Witting 2000, Anon. 2002b). We took a relatively novel approach to investigation of population sub-structure by combining information on regional variation in certain fatty acids

(FA), organochlorines (OC) and heavy metals (Hg and Cd). These substances are presumably acquired from food on the summer feeding grounds and their concentrations reflect long-term accumulation. Hence, the study explores the feasibility of using several different diet-related compounds in *combination* for identification of sub-populations of North Atlantic minke whales. The rationale is that if: (a) minke whales feed little if at all during winter (cf. Horwood 1990), (b) groups of minke whales have a long-term affinity to specific summer feeding grounds, (c) these feeding areas differ substantially in minke whale prey availability and prey choice (Neve 2000, Sigurjonsson et al. 2000, Olsen & Holst 2001, Haug et al. 2002), and (d) the combinations and concentrations of FAs, OCs and heavy metals that are transmitted to the whales via the food differ, it may be expected that this is reflected in different signatures in the whale tissues indicating the existence of ecologically separated groups – or different sub-populations.

In the present study, the same suite of samples from 1998 as used in Hobbs et al. (2003), Møller et al. (2003) and Born et al. (2003) were analysed for regional variation in the *patterns of a combination* of different dietary-related compounds in North Atlantic minke whales. These compounds included eight specific OCs (e.g. PCB congeners, mirex, dieldrin), four unsaturated FAs in blubber, as well as Hg and Cd in muscle, kidney and liver. All are thought to represent long-term accumulation (1+ years) in tissues (Aguilar & Borrell 1988, Norstrom et al. 1992, Dietz et al. 1998, Hickie et al. 2000, Koopman et al. 2002).

A main purpose of the study was to investigate whether the regional variation in this combination of long-term signatures reflect the existence of profound differences in the major marine ecosystems within the geographical range studied; and therefore whether this multi-elemental approach can be used for identification of sub-populations of whales. The four regions considered were: (a) West Greenland, (b) the Central Atlantic represented by whales from the Jan Mayen area, (c) the Northeast Atlantic (Svalbard, Barents Sea, Vestfjorden/Lofoten of coastal Norway), and (4) the North Sea.

### **Ecology of minke whales in the North Atlantic region**

To give the reader a background for the study, the ecology of minke whales in the North Atlantic is briefly summarized.

Apparently, North Atlantic minke whales feed little, if at all, when wintering between about 11° and about 45° N latitude. Pairing likely takes place from December to May, and calving predominantly from October to March, during a period when minke whales are mostly absent from North Atlantic waters. During spring the minke whales migrate north to their boreal, sub-arctic and arctic summer feeding grounds; some, likely few, individuals may stay farther south during summer. There is a tendency that female minke whales summer further north compared to males. Although the whales may occur in areas with deep water in the North Atlantic during summer (e.g.

Anon. 1997), they concentrate on traditional feeding grounds: eastern Canada (Gulf of St. Lawrence, Nova Scotia, Newfoundland-Labrador), off West and Southeast Greenland, around Iceland and Jan Mayen, off Svalbard and in the Barents Sea, off western Norway and in the North Sea (Mackintosh 1965, Jonsgård 1962, 1966, Øien 1988, Larsen & Øien 1988, Horwood 1990, Mitchell 1991, Folkow & Blix 1991, Anon. 1997, Waerebeek et al. 1999).

Within the North Atlantic, no one organism forms the dominant food supply for minke whales (e.g. Skaug et al. 1997). The greater variety of food in the northern hemisphere as compared to that in the southern can be partly attributed to the more complex topography and water conditions in the north (Mackintosh 1965). Although the shallow continental shelf-areas where minke whales feed are areas of great productivity they differ substantially with respect to oceanography (Mann & Lazier, 1991, Anon. 2003, Macdonald et al. 2003): (1) The West Greenland area is influenced by a mixture of waters from the cold East Greenland Current and the warmer and more saline Irminger Current, (2) The East Greenland - Jan Mayen area is dominated by the East Greenland Current that brings cold low-saline polar water south along the eastern coast of Greenland resulting in heavy pack ice almost all year round, (3) the western coast of Svalbard is an area of mixing between polar water and a branch of the warm North Atlantic Current and (4) the Barents Sea is a relatively shallow area that is dominated by the North Atlantic Current. These latter two areas are ice-covered for part of the year. (5) The northwestern coast of Norway is greatly influenced by the North Atlantic Current and the Norwegian Coastal Current resulting in relatively high water temperatures. (6) The North Sea is confined between the British Isles, southern Norway and Denmark, and is influenced by water from the North Atlantic Current as well as land runoff from the surrounding countries. Ice is never present along western Norway and in the North Sea.

These regions also differ with respect to fish and crustacean fauna as reflected in differences among areas in minke whale prey preferences (Folkow et al. 2000, Neve 2000, Anon. 2001, Olsen & Holst 2001, Haug et al. 2002).

## **Materials and methods**

### **Collection of samples**

Tissue samples were available from a total of 159 minke whales that were taken during Greenland and Norwegian licensed whaling operations from 6 May to 31 October 1998 in seven IWC management areas (Fig. 1). The character of the whaling operations determined the sampling areas visited and the aggregate locations within areas exploited by Norwegian whalers (i.e. CM, ES, EB, EC, EN; Fig. 1). However, overall the seasonal and spatial distribution of samples in the present study is representative of the Greenland (cf. Witting 2000) and Norwegian catches in 1998 (Øien, unpublished data). The sam-

ples were analysed for  $^{137}\text{Cs}$  (Born et al. 2002), OC (Hobbs et al. 2003), FAs (Møller et al. 2003), several elements including Hg, Cd and Se (Born et al. 2003), and variation in mitochondrial and nuclear DNA (Andersen et al. 2003). For details on collection of samples and treatment in the laboratory refer to these sources. Because in some cases not all tissues were sampled from each whale, or not all substances were analysed in the individual whale, a total of 104 individual whales (21 M; 83 F) that all had complete data were included in the present analyses (Table 1).

For statistical analyses the data from the various sub-areas were combined into groups that represented the four major marine ecological regimes within the range covered: West Greenland, a Central Atlantic group represented by samples from Jan Mayen (CM) (two samples from East Greenland were omitted); a Northeast Atlantic group that consisted of samples from Svalbard (ES), the Barents Sea (EB) and Vestfjorden/Lofoten (EC) on the northwestern coast of Norway, and the North Sea group (EN); Table 1.

### **Chemical analyses and selection of compounds for statistical analyses**

Cadmium and mercury concentrations in muscle, liver and kidney were included in the present analysis because these elements represent a long-term dietary response (biological half-life 2-30 years; Dietz et al. 1998).

Among 102 PCB congeners and several other OCs in the blubber of the same minke whales (Hobbs et al. 2003), these were selected for the present analysis: PCB153, PCB138 and PCB180, *p,p'*-DDE, HCB, *trans*-nonachlor, mirex and dieldrin. These OCs are known to have long half-lives in mammals (Matthews & Dedrick 1984, Dearth & Hites 1991).

A total of 43 FA have been identified in minke whale blubber (Møller et al. 2003). From among these, four unsaturated FAs found in the outer blubber layer (immediately under the skin) were included in the present study: C14:1n-5, C16:1n-7, C18:1n-9, C20:1n-11. The reasons for selecting these FAs were that (a) FAs in the outer blubber layer are thought to represent a longer-term dietary accumulation than the inner layer, which is more labile (Møller et al. 2003 and references therein, Olsen & Grahl-Nielsen 2003); (b) unsaturated FA generally reflect long-term dietary response better than saturated FA do (Gurr et al. 2002), (c) there was no possibility for transformation of one of these FAs into another by e.g. 2-carbon chain elongation (ibid.), and (d) they were present in a relatively high proportion in all individuals.

Metal concentrations were expressed on dry matter basis ( $\mu\text{g/g}$ ), and OC concentrations on lipid weight basis ( $\text{ng/g}$ ). FAs were expressed as mass percentage of total FAs.

### Statistical methods

Statistical analyses aimed at determining whether the *patterns* of the compounds in combination—relative levels—rather than their individual levels differed from one summering area to another. The statistical package SAS (SAS 1999-2001) was used for all analyses.

A principal components analysis (PCA) of the three groups of variables (heavy metals, OCs, and FAs) gave preliminary insights into their relationships.

Then we explored the correlation between the selected compounds by clustering them based on the correlation matrix (SAS procedure PROC VARCLUS). The number of clusters was determined so that each cluster only had a single eigenvalue greater than one.

Canonical Discriminant Analysis, DCA (procedure PROC CANDIST) was then conducted for all selected metals, OCs and FAs to determine patterns, similarities and differences among the four groups of whales. DCA summarises the data into few canonical variables that captures important differences among sampling locations. The first canonical variable is a linear combination of the compounds that has the highest overall power to discriminate between the groups. The second canonical variable is another linear combination of the compounds, in the sample uncorrelated with the first canonical variable that has the highest possible multiple discrimination between the groups (SAS Institute 1999-2001).

The correlations were explored between the compounds and the canonical variables together with the standardised (mean=0, SD=1) canonical coefficients. The correlation coefficients measure the univariate relationship between the compounds and the canonical variable, while the standardised coefficients show the contribution of the compounds in the presence of each other compound and therefore provide a multivariate approach to interpretation of the contribution of the variables acting in combination (Rencher 1995).

The canonical variables were then used to determine the ability to assign the whales to the four areas (procedure PROC DISCRIM). Based on the generalised squared distance function each whale was placed in the area from which it had the smallest distance. This discrimination was then validated by assigning the single whale based on the discrimination function calculated from all the other whales, and by repeating this procedure for each whale.

Analyses of variance (ANOVA) followed by Tukey *post hoc* tests were used to test for differences in mean canonical variables between areas and sexes.

## Results

### Correlations

Correlation analyses showed a general structure in the data. Mercury (Hg) in muscle, liver and kidney was highly inter-correlated, and (weakly) with cadmium (Cd) in liver and kidney. Cd in muscle, however, was negatively correlated with Hg in all tissues and uncorrelated with Cd in other tissues. Organochlorines were positively correlated with one another - except that dieldrin was negatively correlated with mirex - and with mercury in all tissues; they were negatively correlated with Cd in muscle and overall uncorrelated with Cd in other tissues. The fatty acid C16:1n-7 showed a striking negative correlation with all OCs and with mercury; C18:1n-9 showed a similar pattern but the correlations were much weaker. The other two FAs were positively correlated with OCs and metals.

### Principal component analyses

Preliminary PCAs performed separately for the different groups of compounds (metals, OCs and fatty acids) confirmed these relationships between the variables. For the metals, the first component (explaining 45% of the total variation) was positively correlated with all metals except for Cd in muscle, and the second component (explaining 26%) was positively correlated with Cd in liver and kidney and weakly negatively correlated with Hg in all tissues. The PCA of OCs showed that all compounds were positively correlated with the first component (explaining 60%) although dieldrin had a relatively low correlation. The second component (explaining 17%) was strongly positively correlated with *trans*-nonachlor and dieldrin and negatively correlated with mirex and HCB (hexachlorbenzene). The PCA of fatty acids showed that C14:1n-5, C16:1n-7 and C18:1n-9 were positively correlated and C20:1n-11 negatively correlated with the first component (explaining 49% of the total variation). The second component (explaining 28%) was mostly positively correlated with C14:1n-5 and C20:1n-11.

### Cluster analysis

The clustering of the compounds based on the correlation matrix resulted in five clusters that explained 70% of the total variance (Table 2). Cluster 1 that explained 36% of the total variance consisted of all the OCs, except dieldrin. Relatively high values of squared correlations with their own cluster together with low values of squared correlations with the next closest cluster indicated that these variables were well separated by this cluster. Low values of the indicator "1 minus these squared correlations" also indicated good separation (Table 2). PCB180 and PCB153 showed the highest separation from the other clusters while *trans*-nonachlor showed the lowest separation. Cluster 2 explaining 12% of the total variation was composed of Hg in kidney, muscle and liver, and two FAs (C16:1n-7 and C20:1n-11). Hg in the three tissues showed the highest separation by this



cluster ( $r^2 \geq 0.79$ ), and the two FAs the lowest ( $r^2 \leq 0.50$ ). Cluster 3 explained only 9% of the total variation and was composed of Cd in kidney and liver and the OC, dieldrin. Cadmium in kidney and liver were well separated by this cluster ( $r^2 \geq 0.71$ ), whereas dieldrin was not ( $r^2 = 0.20$ ). Cluster 4 was composed of the two other FAs (C18:1n-9 and C14:1n-5). Cluster 5 consisted only of cadmium in muscle, which was shown to be anomalous in the earlier correlation analyses. In each of the first 3 clusters there were variables that fitted poorly into the cluster: *trans*-nonachlor in cluster 1, the two FAs in cluster 2, and dieldrin in cluster 3.

These results of the cluster analysis were consistent with the PCA of groups of variables. Thus, Cd in muscle had an anomalous loading on the first PC for metals, and fell into a separate cluster, and the different loadings of Cd and Hg in the two components agreed with the separation of Hg and Cd into different clusters. The different loading of dieldrin in the PCA was also consistent with its distinct clustering.

### Canonical discriminant analyses

CDAs were performed for four areas (West Greenland, Jan Mayen, the Northeast Atlantic and the North Sea) for each sex separately. However, the discrimination ability was no higher than when the two sexes were pooled. The three canonical variables explained 61, 26 and 13% of the variation, and all were significant at the 1% level.

The first canonical axis separated the North Sea (highest mean CAN1) from Jan Mayen and the Northeast Atlantic (in-between values of CAN1), which again were separated from West Greenland (lowest mean CAN1) (Fig. 2A). The first canonical variable varied significantly between areas (ANOVA,  $F = 75.9$ ,  $P < 0.0001$ ), but the difference between the Central and the Northeast Atlantic did not make much contribution to this variation (Tukey *post hoc* test, significance level of 5%).

The fatty acids C14:1n-5, C16:1n-7 and C20:1n-11, Cd in liver and Hg in muscle, and HCB and mirex contributed most to the first canonical variable judged from the standardised coefficients (1.10, -0.93, -0.74, -0.83, 0.79, -0.81 and 0.70, respectively) (Table 3). These compounds were all moderately correlated with the first canonical variable ( $r$  between 0.20 and 0.57), except for C20:1n-11 ( $r = 0.06$ ); Table 3. This means that a whale with a high value of CAN1 (North Sea) had a relatively high concentration of compounds with high positive standardised coefficients (C14:1n-5, Hg in muscle, mirex and PCB153) and a relatively low concentration of compounds with high negative standardised coefficients (C16:1n-7, C20:1n-11, Cd in liver, HCB and PCB180). A whale with a low value of CAN1 (West Greenland) had the opposite concentration pattern.

The compounds C18:1n-9, PCB180, PCB153, dieldrin and Hg in muscle contributed most to the second canonical variable (standardised coefficients: -0.98, -0.87, 0.79, 0.55 and 0.53, respectively; Table 3). Among these, C18:1n-9 showed the highest correlation coefficient ( $r =$

-0.80). The second canonical variable separated the minke whales from Jan Mayen plus the Northeast Atlantic (lowest mean CAN2; relative high concentration of C18:1n-9 and PCB180 and relative low concentration of PCB153, dieldrin and Hg in muscle) from those from West Greenland (in between mean CAN2), which again was separated from whales from the North Sea (highest mean CAN2) (Fig. 2A). The second canonical variable varied significantly among areas except, again, between Jan Mayen and the Northeast Atlantic (ANOVA,  $F = 32.5$ ,  $P < 0.0001$ , followed by Tukey *post hoc* test, significance level of 5%).

The third canonical variable separated Jan Mayen from all the other areas (Fig. 2B, ANOVA,  $F = 15.5$ ,  $P < 0.0001$  and Tukey *post hoc* test), including the Northeast Atlantic. Hg in kidney, *p,p*-DDE, C14:1n-5, C18:1n-9 and C20:1n-11 contributed most to the third canonical variable (standardised coefficients: 0.90, 0.69, -0.67, 0.63 and 0.61, respectively). Hg in kidney had the highest correlation coefficient ( $r = 0.57$ ), whereas *p,p*-DDE was only weakly correlated ( $r = 0.05$ ) with the third canonical variable.

In none of the areas did the three canonical variables (CAN 1-3) differ significantly between sexes (two-way ANOVA with the factors "area" and "sex", nested "area"; CAN1:  $F = 1.18$ ,  $P = 0.33$ , CAN2:  $F = 0.65$ ,  $P = 0.63$ , CAN3:  $F = 1.53$ ,  $P = 0.19$ ).

### Assignment test

During the assignment test based on the transformations developed by the CDA, about 84% (87 of 104) of the individual whales were classified to the area where they had been caught (Table 4). The most common mis-assignment was between the Jan Mayen and the Northeast Atlantic areas. When the assignment of each whale was based on the discrimination function derived from the other whales, the fraction of correctly classified whales was about 67% (70 out of 104). Again the most common mis-classifications were between Jan Mayen and the Northeast Atlantic.

## Discussion

The present study hypothesised that several dietary-related compounds reflected the existence of four markedly different North Atlantic marine environments where minke whales feed during summer. The study tested (a) whether variation in *patterns* of these compounds that have different origin and ecological and physiological pathways could identify different groups of minke whales with long-term affinity to these areas, and therefore (b) whether the multi-elemental approach is useful for discrimination of sub-populations - or ecologically separated groups of whales.

Basically the multi-elemental analyses supported the results of the genetic study (Andersen et al. 2003). It was therefore concluded that ecological markers can assist in identification of sub-populations and

can be particularly useful in lack of other evidence of stock separation.

However, premises for this method to be useful are: (1) Within the range explored there must exist profound regional variation in the compounds studied (or the combination of compounds), (2) this variation must also be expressed in different minke whale food, and (3) be present in different tissue signatures (i.e. the signal in the whale must be retained over several years).

The spatial occurrence of Cd, Hg and OCs, and their levels and patterns, in the North Atlantic marine ecosystems result from complex processes that differ from compound to compound. In the North Atlantic, both Cd and Hg originate from long-distance transportation of anthropogenic emissions, or from natural sources influencing on the "local" environment (Dietz et al. 1998). Concentrations of these heavy metals in the marine biota vary on a regional scale. Hg and Cd in liver of the relatively stationary (at least in contrast to minke whales) ringed seal (*Phoca hispida*) showed significant regional differences among West Greenland, East Greenland, Svalbard and the White Sea. Generally, concentrations were highest in Greenland (Riget et al. 2005).

OCs are solely of anthropogenic origin and are mainly brought to the Arctic via long-range transportation in the atmosphere or oceans. However, in areas such as the North Sea that are closer to urbanized areas local sources may also be important. Differences in concentrations of PCBs, DDT and chlordanes related compounds have been observed between west Greenland, east Greenland and Svalbard in several arctic species including ringed seals and beluga, *Delphinapterus leucas* (Muir et al. 2000, Cleeman et al. 2000, Andersen et al. 2001), polar bears, *Ursus maritimus* (Norstrom et al. 1998), and seabirds (de Wit et al. 2004). Higher concentrations of all three OCs were generally found in biota from Svalbard and the east Barents Sea than west Greenland. This appears to reflect the influence of European and Russian sources on the Barents Sea and southern Kara Seas (de Wit et al. 2004, Norstrom et al. 1998). Higher levels of PCBs and DDT have also been found in Atlantic cod (*Gadus morhua*) from the North Sea compared to those from Iceland (Stange & Klungsøyr 1997). Taken together all this information suggests that there is a gradient of PCB and persistent OCs across the North Atlantic from the North Sea to Greenland, and from the Barents Sea to Greenland, which could influence levels in minke whale tissues. Therefore, it would be reasonable to hypothesize that minke whales feeding in the eastern portion of the North Atlantic minke whales summer range could differ significantly in levels and patterns of PCB congeners compared to those feeding in western Greenland.

Mercury and OCs are known to bio-magnify and therefore the load of these pollutants increase along the food chain (cf. AMAP 1998, Anon. 2002c). Although some studies have indicated that Cd biomagnifies (e.g. Dietz et al. 1996), there is little evidence that Cd biomagnifies when the entire food web is considered and the study by Campbell et

al. (2005) found biodilution of Cd (i.e. a decrease in concentration of an element with increasing trophic level). Minke whales in a feeding area probably act as selective (through their feeding preferences, e.g. piscivory versus carcinophagy) integrators of the occurrence of the compounds in that area.

FAs have been used as a tool to discriminate between populations of various marine mammals (reviewed in Møller et al. 2003) including minke whales (ibid., Olsen & Grahl-Nielsen 2003). FA composition in the blubber reflects not only the feeding preferences of the minke whales but also their ability to synthesise and modify FAs (Møller et al. 2003). Nevertheless, the variations in FA signatures in the outer blubber layer in minke whales from different areas of the North Atlantic are believed to reflect regional differences in types of food available to the whales (ibid.).

The regions studied differ with respect to occurrence of types of minke whale prey. Capelin (*Mallotus villosus*) and sand eel (*Ammodytes* ssp.) are important food for minke whales in West Greenland waters whereas polar cod (*Boreogadus saida*) seems to be of greater importance in the East Greenland region (reviewed by Neve 2000). During the last decade or so, Atlanto-boreal species like Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*), herring (*Clupea harengus*), mackerel (*Scomber scombrus*) have either not been present in Greenland waters or have occurred there in such low numbers that they have been insignificant as minke whale food (e.g. Anon. 2001). Krill (*Thysanoessa* sp.) and herring are two of the most prominent prey items in the diet of minke whales in the Northeast Atlantic where gadoid fish (cod, saithe, haddock) are also important prey (reviewed by Haug et al. 2002). Within the NE Atlantic area there are regional differences in prey preferences. Consumption of herring is almost exclusively confined to the Barents Sea and the northwestern coast of Norway whereas consumption of krill is more pronounced in the Svalbard area (Folkow et al. 2000, Haug et al. 2002). Herring is a predominant food item in the Norwegian Sea whereas sand eel dominates the minke whale food in the North Sea. In this latter area, mackerel and other fish (e.g. herring) constitute the remainder of food items (Olsen & Holst 2002). Sand eel and herring are important minke whale food at Scotland (Macleod et al. 2004). It is highly likely that the prey species synthesize and accumulate the various compounds differently and therefore that regional variation in minke whale prey preferences will reflect such differences.

A preliminary exploration of the correlation structure of the selected compounds by cluster analysis separated seven out of eight OCs into one cluster (Table 2). Highly chlorinated PCB congeners and DDE are known to be often highly correlated in marine mammals (e.g. Weisbrod et al. 2000).

Hg in muscle, liver and kidney also separated into one cluster, which was also the case with Cd (Table 2). High inter tissue correlations of both mercury and cadmium have often been observed. In animals like minke whales that feed on both fish and crustacean, Hg and Cd

concentrations may be negatively correlated (Riget & Dietz 2000). Hg is known to be present in high concentrations in fish relative to Cd concentrations, whereas the opposite is the case in crustaceans (Dietz et al. 1996).

The variable clustering showed clear separation between OCs as a cluster, mercury in all tissues, and Cd in liver and kidney. However, the correlation analyses also showed that these groups of variables were not independent of one another: there could be identified a general “contamination” signature containing all the OCs and mercury. Cadmium, on the other hand, was not part of this signature and if anything was negatively associated with it. The first two canonical variables had generally positive correlations with the OCs and mercury, and negative correlations with cadmium in kidney (CdK) and liver (CdL), and were principally distinguished by having opposite correlations with cluster 4: C18:1n-9 and C14:1n-5. The third canonical variable was distinctive in having only weak correlations with all the OCs, but positive correlations with Hg and with CdK and CdL. Therefore combining the signals of the compounds that have different ecological and physiological path-ways into one analysis is expected to be a stronger tool for separation of groups of minke whales than using the groups of variables in isolation as done in Hobbs et al. (2003), Born et al. (2003) and Møller et al. (2003).

Hobbs et al. (2003) used OCs to infer stock structure of North Atlantic minke whales. They found differences among areas in concentrations of certain OCs and suggested that West and Southeast Greenland whales were distinct from whales from Jan Mayen, the Northeast Atlantic and the North Sea. However, principal component analyses (PCA) including a total of 71 PCBs and 20 OC pesticides did not reveal any very distinct groupings of animals based on variation in contaminant patterns by region. Møller et al. (2003) studied the regional variation in 43 fatty acid composition in both deep and superficial blubber. From this analysis, the existence of three regional stocks was inferred: West and East Greenland, the Northeast Atlantic (Jan Mayen, Svalbard, Barents Sea, Vestfjorden/Lofoten) and the North Sea. Using regional variation in concentrations of mercury, selenium and cadmium in various tissues, Born et al. (2003) found significant differences in a least one long-term diagnostic element between several areas. PCAs on 19 elements in baleen suggested that four groups of whales could be distinguished: West Greenland, Jan Mayen, Northeast Atlantic (Svalbard, Barents Sea, Lofoten/Vestfjorden), and the North Sea.

In contrast to the studies by Hobbs et al. (2003), Møller et al. (2003) and Born et al. (2003), the present study only included substances that were thought to represent long-term deposition in tissues and hence likely reflect long-term affinity to a particular summer feeding ground. Furthermore, our study explored the combined difference reflected in compounds of different origin.

All the canonical variables of the CDA reflected complex combined patterns of the three groups of compounds involved and each ca-

nonical variable included substances of importance from different groups. However, while the substances within each group were correlated, the correlations were not perfect, and so the canonical variable had different loadings on the different members of each group. The ecological or physiological interpretation of the specific composition of the canonical variables is very difficult because of the highly different nature and pathways of the compounds involved. We are not able to offer any satisfactory physiological or ecological interpretation of the results of the CDAs.

Different OCs and heavy metals had different loadings and therefore the differences detected did not reflect a simple picture of regional variation in pollution. The first two canonical variables differed in having opposite correlations with cluster 4 fatty acid variables, so there seemed to be some sort of food level separation involved.

The use of multi-elements is valuable, because each group of variables tends to be correlated, but we see for example that both the first and the second canonical variable reflected a possible "contamination" signature in the same way, but perhaps differed on the fatty-acid signature, while the third canonical variable revealed differences in metal signatures.

The ability of the canonical variables to discriminate among the whales from the four areas where they were caught was relatively good (84% correctly assigned). However, cross validation of the discrimination success rate by analysing the sensitivity of each whale to the discrimination reduced the success rate to about 68%. To some extent this reflected the sensitivity of the test to small sample size.

A canonical discrimination procedure on OC concentrations has been used to separate "stocks" of beluga whales in eastern Canada and western Greenland with a success rate of 93% (cross validation success rate of 89%) (Innes et al. 2002). However, Innes et al. (2002) included a total of 49 OC congeners in their analyses and did not specifically select those that are likely to represent long-term deposition, and therefore long-term affinity to a certain area. Hence, the classification in Innes et al. (2002) of belugas to an area of catch inevitably would have a higher precision, but the groups or "stocks" identified by including also short-term dietary-related OC congeners may more arbitrarily reflect a local and short-term signal and not necessarily stable sub-populations.

In the present study, the most common mis-classifications were of whales from the Jan Mayen area to the Northeast Atlantic, and *vice versa*, which is consistent with generally poor discrimination of these two groups in the CDA. This may have been caused by several factors: (1) that Northeast Atlantic represented a mixture of whales from Svalbard, the Barents Sea and Vestfjorden/Lofoten, or (2) that Jan Mayen and Northeast Atlantic whales belong to the same group of whales. The study by Andersen et al. (2003) indicated that whales from Jan Mayen (and eastern Greenland) were genetically distinct from those in the Northeast Atlantic region (Svalbard, Barents Sea,

Vestfjorden/Lofoten). However, when analysed separately, whales from Jan Mayen, Vestfjorden/Lofoten, Svalbard, the Barents Sea and the North Sea did not differ significantly at the mtDNA level whereas at the nuclear DNA level (microsatellites) whales from Jan Mayen differed from those sampled at Svalbard (Andersen et al. 2003). The OC levels in whales from Jan Mayen did not differ significantly from those in whales from Svalbard, Barents Sea and Vestfjorden/Lofoten. Furthermore, FA signatures did not differ among Jan Mayen, Svalbard, Barents Sea and Vestfjorden/Lofoten (Møller et al. 2003). Hence, also when analysed separately the dietary-related compounds included in the present study did not find a clear distinction between Jan Mayen and Northeast Atlantic minke whales. This lack of a clear distinction, and low sample size, likely explain the relatively high mis-classification rate found in the present study between these two areas.

Based on genetic analyses and analyses of stock boundaries using the Boundary Rank Method, the IWC working group on North Atlantic minke whales concluded in 2004 that (1) genetic studies have confirmed a distinction between the Central and Northeast Atlantic, and (2) that there is little or no evidence for distinction between whales from the Vestfjorden/Lofoten area and from the waters surrounding it (Anon. 2004). These conclusions are not contradictory to the findings in the present study. However, there were indications of a further subdivision of the group of minke whales in the Barents Sea (Anon. 2004).

Several studies have shown differences in the concentrations of OCs related to sex in minke whales (Kleivane & Skaare 1998, Hobbs et al. 2003) and in other baleen whales (Aguilar & Borrell 1998). Sex differences were therefore expected to influence the canonical discriminant analysis, however, this was not the case, probably because the canonical discriminant analysis is more sensitive to changes in ratios than in levels in terms of concentration.

Various elements deposited in baleen (Born et al. 2003) and  $^{137}\text{Cs}$  in muscle (Born et al. 2002) could have been included in the analyses because they represented a relatively long time dietary response. However by also including these elements the number of whales available for the analysis would have been too small. The reason being that the basic criterion was that all 14 compounds should have been analysed in each individual whale.

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# Table captions

## Table 1.

Number by area and sex of North Atlantic minke whales sampled in 1998 that were included in the analyses of regional differences in various dietary-related compounds. For explanation of acronyms see Fig. 1.

## Table 2.

Results of clustering various compounds in tissues of 104 minke whales that were sampled in the North Atlantic in 1998. The clustering was based on the correlation matrix (see Material and methods). K = Kidney; M = Muscle; L = Liver.

## Table 3.

Correlation coefficients and standardised (mean=0, SD=1) canonical coefficients between canonical variables (CAN1-3) and various compounds in 104 minke whales that were sampled in the North Atlantic in 1998. K = Kidney; M = Muscle; L = Liver.

## Table 4.

Results of the assignment of 104 individual minke whales to four areas in the North Atlantic based on the three canonical variables and a cross validation of the assignment.

Numbers of correctly assigned specimens are shown in bold. For location of areas and explanation of acronyms see Figure 1.

## Figure captions

Figure 1.

Map showing the location of sampling of tissues from 159 minke whales (125 F, 34 M) at seven North Atlantic summer feeding grounds in 1998. A sub-set of 104 of these whales was included in the present paper (Table 1). Boundaries of the International Whaling Commission (IWC) North Atlantic management areas (Anon. 1993) are shown. Approximate minke whale summer range (Stewart & Leatherwood 1985, Donovan 1991a,b, Anon. 1997) is indicated in darker grey. IWC acronyms of different management areas are: WC (West, Canada), WG (West, Greenland), CG (Central, Greenland), CIC (Central, Island, Coastal), CIP (Central, Iceland, Pelagic), CM (Central, Jan Mayen); ES (East, Svalbard), EB (East, Barents Sea), EC (East, Coastal), EN (East, North Sea).

Figure 2.

Mean and SD by sampling region of the two first canonical variables (A) and the first and the third canonical variables (B) based on 18 different compounds (cf. Table 2) in tissues of 104 minke whales that were sampled in the North Atlantic in 1998. WG = West Greenland, CM = Jan Mayen, NE = the Northeast Atlantic (Svalbard, Barents Sea and Vestfjorden/Lofoten), EN = the North Sea.

Area	Acronym	Number of specimens		Period of collection
		Females	Males	
West Greenland	WG	19	6	6 May - 31 October
Jan Mayen	CM	16	3	7 June - 1 July
Northeast Atlantic	(NE) <sup>1</sup>	39	5	15 May - 14 August
North Sea	EN	9	7	15 May - 8 June
Total		83	21	

1) Consisting of whales from Svalbard, ES (F:M 13:1), the Barents Sea, EB (23:1) and Vestfjorden/Lofoten, EC, in NW Norway (3:3)

**Table 1**



		Squared correlation with			Cumulative proportion of variance explained
Cluster	Compound	Own cluster	Next cluster	(1-own cluster)/ (1-next cluster)	
1	PCB153	0.89	0.17	0.13	0.36
	PCB138	0.70	0.26	0.41	
	PCB180	0.93	0.20	0.09	
	<i>p,p'</i> -DDE	0.71	0.12	0.33	
	<i>trans</i> -nonachlor	0.54	0.28	0.64	
	HCB	0.74	0.04	0.27	
	Mirex	0.70	0.09	0.34	
2	HgK	0.79	0.23	0.27	0.48
	HgM	0.84	0.16	0.19	
	HgL	0.79	0.12	0.24	
	C16:1n-7	0.50	0.16	0.60	
	C20:1n11	0.35	0.04	0.68	
3	CdK	0.75	0.01	0.25	0.57
	CdL	0.71	0.02	0.29	
	Dieldrin	0.20	0.09	0.88	
4	C18:1n-9	0.73	0.03	0.27	0.65
	C14:1n-5	0.73	0.01	0.27	
5	CdM	1.00	0.03	0.00	
Total					0.70

**Table 2**

Compound	Cluster	Correlation coefficients			Total standardised coefficients		
		CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
PCB153	1	0.364	0.226	-0.116	0.403	0.788	-0.412
PCB138		0.411	0.257	-0.120	0.219	-0.442	-0.412
PCB180		0.376	0.193	-0.053	-0.611	-0.87	0.160
<i>p,p</i> -DDE		0.303	0.234	0.053	-0.154	-0.063	0.692
<i>trans</i> -nonachlor		0.535	0.369	0.025	0.668	-0.345	-0.109
HCB		0.152	0.244	-0.082	-0.811	0.427	0.071
Mirex	2	0.204	0.212	-0.02	0.703	0.267	-0.352
HgK		0.416	0.419	0.568	0.027	0.190	0.901
HgM		0.569	0.354	0.526	0.793	0.531	0.295
HgL		0.329	0.300	0.470	-0.331	-0.078	-0.229
C16:1n-7		-0.446	-0.398	-0.178	-0.929	0.095	0.295
C20:1n-11		0.055	0.604	0.273	-0.736	0.337	0.608
CdK	3	-0.191	-0.199	0.263	-0.012	-0.123	0.062
CdL		-0.390	-0.235	0.234	-0.826	-0.190	0.169
Dieldrin		0.207	0.408	-0.240	-0.256	0.554	-0.469
C18:1n-9	4	0.264	-0.800	0.165	0.136	-0.975	0.631
C14:1n-5		0.361	-0.270	-0.145	1.098	0.059	-0.674
CdM	5	-0.279	-0.073	-0.169	-0.051	0.005	-0.249

**Table 3**

	Assignment				Cross-validation			
Area	West Greenland WG	Jan Mayen CM	NE Atlantic NE	North Sea EN	West Greenland WG	Jan Mayen CM	NE Atlan- tic NE	North Sea EN
WG	<b>23</b>		2		<b>22</b>	1	2	
CM		<b>16</b>	3		2	<b>10</b>	6	1
NE		6	<b>34</b>	4	2	8	<b>29</b>	5
EN			2	<b>14</b>		3	4	<b>9</b>
Total	23	22	41	18	26	22	41	15

**Table 4**

Figure 1.

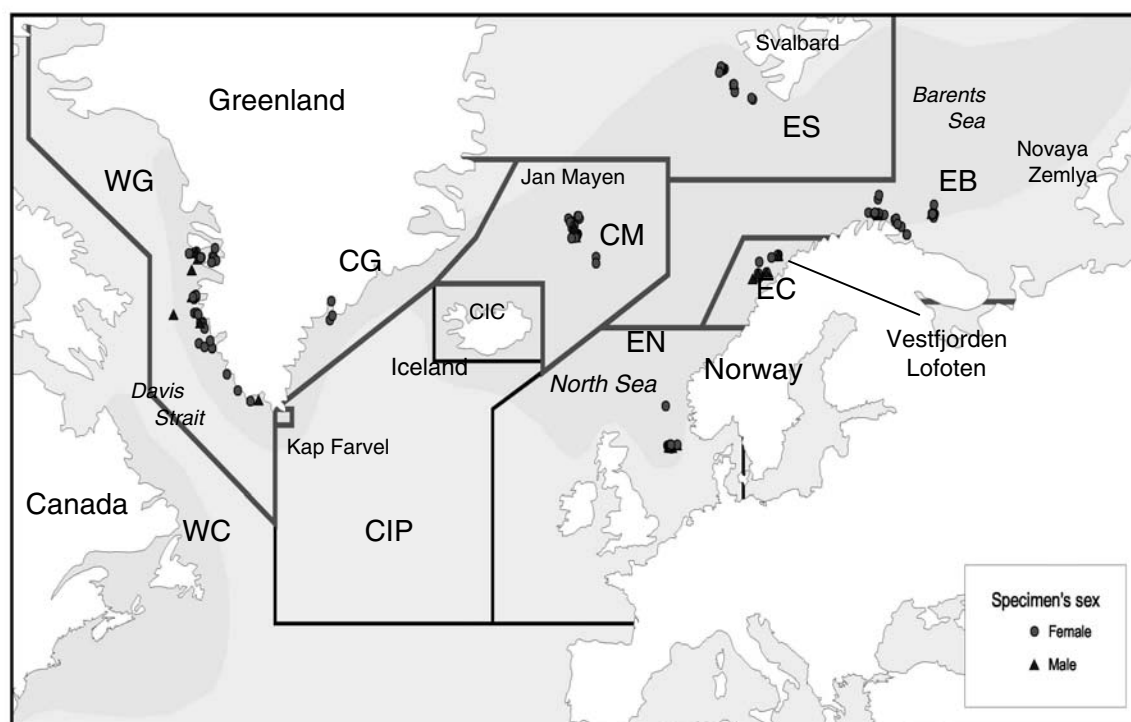
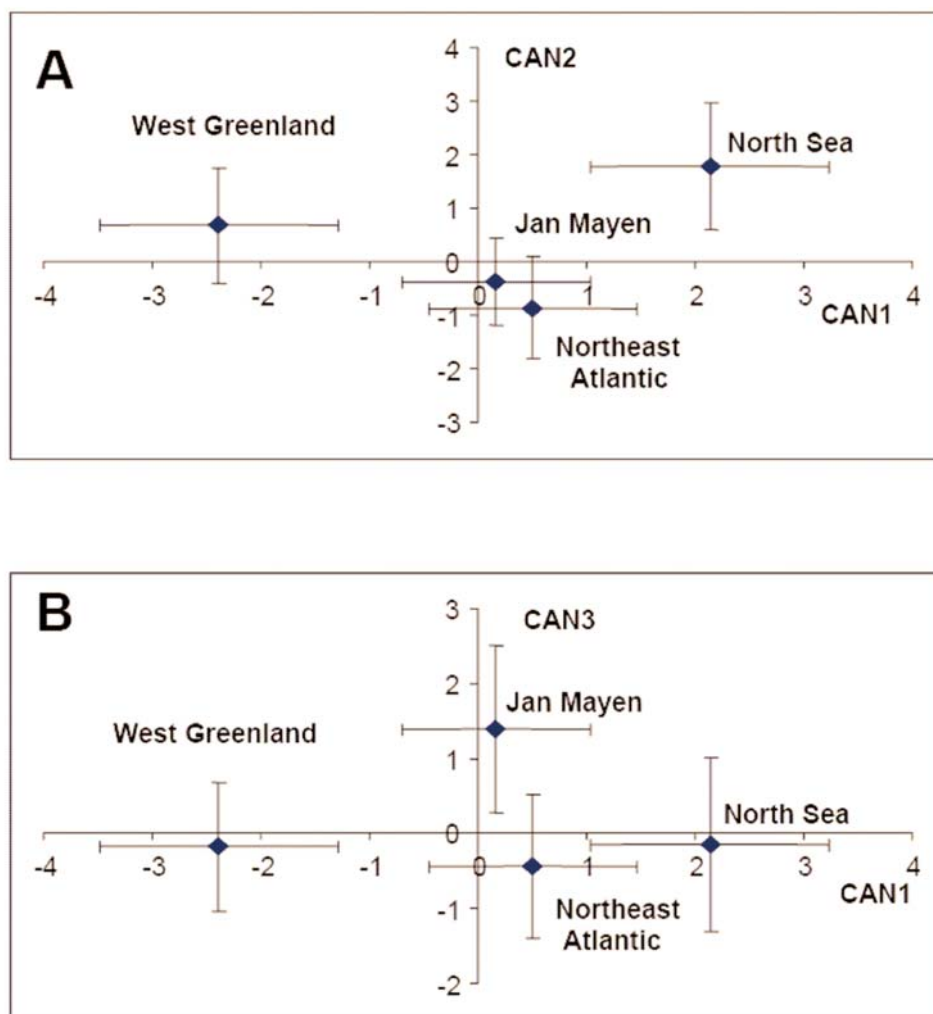


Figure 2.

WB MS 04-46 Born et al. Minke whales

Fig. 2



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## **Nutritional lipid quality of West Greenland marine species**

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Møller P., Johansen P. and Hellgren L. I.

In preparation.

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# Nutritional lipid quality of West Greenland marine species

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## Abstract

The population of Greenland has recently gone through a rapid change in diet, moving away from a traditional marine diet to a more western-like diet, based on imported foodstuffs. This dietary transition is mainly driven by the general socio-cultural changes linked to a more western-like life-style, but awareness of contamination of the marine food sources may also decrease the intake of the traditional food items. The marine diet exposes Greenlanders to a high intake of heavy metals and persistent organic contaminants, but it has also health-promoting properties, in particular through the high intake on n-3 fatty acids. Hence, the dietary transition leads to a decrease in intake of contaminants, but also a concomitant decrease in n-3 fatty acids. Due to this "Arctic dilemma", it is essential that we can point at food-items with a superior nutritional lipid quality and a relatively low contaminant load. Therefore, we report on the lipid quality based on the content of essential fatty acids (EFA) and other bioactive fatty acids, as well as the content of and balance between fatty acid classes in blubber and/or muscles from species particular importance in the traditional Greenlandic diet, and compare these data with the published contaminant content. All of the 29 species investigated had a high nutritional lipid quality, with potential positive implications to public health in West Greenland. The most favorable fatty acid composition, with low levels of SFA and high levels of MUFA and PUFA were observed in invertebrates, lean fish and blubber. As expected, all dietary components represented a favorable balance between n-6 PUFA and n-3 PUFA with a ratio of 1 or less. When calculating the mass of n-3 PUFAs per kg wet weight, harp seal and ringed seal blubber were clearly the best sources. We have also, for the first time, identified several food items in the traditional diet with relative high concentrations of the highly bioactive fatty acids pristanic and phytanic acid. The richest sources were hooded seal and beluga blubber. Levels of these fatty acids varied substantially between species within as well as between taxa. Based on our data on lipid quality components (LQCs) and data on contaminant content, we suggest that con-



taminant-corrected LQCs are used as a tool to improve the dietary recommendation in societies with a traditional high intake of marine resources. Using this concept, we have picked a number of sources with the highest LQC to contaminant (i.e. Hg and PCB) ratio. These data will be important when giving dietary recommendations, balancing the risk from the contaminants and the health-promoting fatty acids in the traditional diet.

**Key words:** Fatty acid composition, LA, LNA, EPA, DHA, Phytanic acid, Pristanic acid, MUFA, n-3 PUFA, n-6 PUFA, n-6/n-3, Lipid content, Marine resources, Traditional diet, West Greenland

## Introduction

Lipids of marine origin have traditionally been the major energy source in the diets of the indigenous people in the Arctic and sub-Arctic areas of Greenland and North-America [1, 2]. The fatty acid composition of these diets are characterized by low levels of saturated fatty acids (SFA) and n-6 polyunsaturated (n-6 PUFAs), and high levels of monounsaturated (MUFA) and long-chained n-3 polyunsaturated fatty acids (n-3 PUFAs), compared with the western diets.

However, the dietary habits of these peoples are at present in a rapid transition, going from the marine traditional diets, based in local culture and traditions, to a western diet, based on imported food-stuffs [3]. In a recent comparison of the dietary habits in Uummannaq town in northern Greenland in 1974 and in 2004, it was shown that traditional food accounted for about 40 % of the energy intake in 1974 but only 20 % in 2004 [4]. The rapid rate with which the dietary transition occurs is illustrated by the pronounced differences in the food-pattern between the generations. Thus, in one study from the mid 1990th, 18–24 year old Inuit Greenlanders had 20 traditional meals/month, while the group of over 60 year olds consumed more than 40 meals/month [5]. A similar pattern has also been reported in Canadian studies [6].

This dietary transition is mainly driven by the general socio-cultural changes towards a western life-style in the indigenous societies, but the focus on the potential health-risk linked to high concentrations of some contaminants in some marine diet items may further have decreased the consumption of these foodstuffs. In several studies, a close correlation between the concentration of persistent organic pollutants in the blood, and the intake of traditional diets has been established [7]. In Greenland the traditional diet is still valued by people, but may result in a very high intake of contaminants. Thus from a dietary survey in the Disko Bay region in West Greenland, Johansen *et al.* calculated that in average this population exceeded "acceptable/tolerable intakes" for a number of contaminants (cadmium, mercury, PCBs, chlordanes and toxaphene) by a factor of between 1.5 and 6 [8]. Dietary sources were mainly tissues from seals and whales (cadmium from liver and kidney, mercury from meat and

liver, and organochlorines from blubber). It therefore could be recommended to avoid these tissues as part of the diet.

However, basing dietary recommendations regarding the traditional diet solely on the potential health-risk due to its content of contaminants would be inappropriate, since the potential health-promoting properties of this diet also have to be taken into account. Thus, the traditional marine diet is very rich in the long-chained n-3 polyunsaturated fatty acids (n-3 PUFA), compared to the western diet [9], and as a consequence, the traditional food-pattern leads to a high plasma concentration of these fatty acids and a relatively low concentration of the long-chained fatty acid of the n-6 series (n-6 PUFA) [10]. Therefore, the transition to a western-type diet leads to decreased level of plasma n-3 PUFAs, and an increased ratio between n-6/n-3 PUFAs, [4], as well as an increased intake of saturated fatty acids [4, 6].

Ever since the seminal work of Bang and Dyerberg on the relationship between the high intake of n-3 PUFA and low prevalence of cardiovascular diseases among the Greenlanders [11], it has been acknowledged that long-chained n-3 PUFA may reduce the risk of developing cardiovascular diseases [12]. This observationally based assumption now has been verified in several clinical interventions studies, and most meta-analyses of the published randomized controlled trials show that increased intake of n-3 PUFAs (as fatty fish or as dietary supplement) reduces the risk of sudden cardiac death and the risk of a fatal myocardial infarction with 20-25 % in patients that have had a non-fatal myocardial infarction or were diagnosed with angina [12, 13]. Furthermore, a meta-analysis of randomized controlled-trials on the effect of n-3 PUFA on blood-pressure, has shown that daily n-3 PUFA supplements reduce both systolic and diastolic blood pressure, when given in amounts similar to the average intake in a recent double portion study from Greenland [4, 14].

Thus, the transition from a n-3 PUFA rich traditional diet to a typically n-3 PUFA-poor western diet is expected to have a negative impact on cardiac health. The negative health-effects of the dietary transition must be expected to be amplified through the general alteration in lifestyle, going from a hunter-gatherer subsistence with intense physical activity to a more sedentary western-type of salary-based economy. In a recent study among Alaskan Inuit, n-3 fatty acids in plasma were inversely correlated to glucose-tolerance, insulin-resistance, plasma triacylglycerols and diastolic blood pressure indicating the prevalence of the metabolic syndrome to be negatively correlated to the intake of traditional foodstuffs [15]. Hence, advocating for a general decreased intake of marine food-stuff in the Arctic and sub-Arctic due to the health-hazards from contaminants also means advocating for a diet that is likely to enhance the negative impact of a more sedentary, western life-style.

However, this "Arctic dilemma" can be solved, if it is possible to identify components of the Arctic traditional diet that are rich in n-3 PUFAs and other bioactive components without having high con-

centrations of contaminants. Significant amounts of data on the concentration of different contaminants in Greenlandic diet are available [16], but at present it is not possible to make specific dietary recommendations in which the risk of contaminants are balanced against the health-promoting lipid composition, since we lack the detailed information on the lipid content and composition in the different dietary components. This motivated us to perform this study in which we have analyzed the fatty acid composition and lipid content of muscle/soft tissue and blubber of 29 marine key species of particular importance to the traditional diet of people in West Greenland. We report on the lipid quality of these marine resources based on the content of essential fatty acids (EFA) and other fatty acids of nutritional importance, as well as the distribution between the different fatty acid classes.

## Materials and Methods

### Field collection and sampling

Sampling was organised and performed as part of a larger collection scheme related to the projects MARFAT (NERI, Denmark) and MAR-TOP (GINR, Greenland) with a focus on the marine ecosystem of West Greenland. Details on location and sampling procedures are described elsewhere [17].

### Field collections

In total, 29 species were included in our analysis (Table 1). Invertebrates, fish and seabirds were sampled between 62° and 69°30'N and marine mammal species up to 71°30'N. Twenty of the 29 species (i.e. all invertebrate and fish species, 1 out of 5 seabird species, 2 out of 7 marine mammal species) were collected in July-September 2003, whereas the remaining 4 species of seabirds were sampled in March 2003 (little auk), November 2003 (Brünnichs guillemot) and January 2004 (common eider and king eider). The remaining 5 species of marine mammals were sampled from November 2000 to May 2004. Samples of most of the marine fish species as well as Northern shrimp (*Pandalus borealis*), snow crab (*Chionoecetes opilio*) and minke whale (*Balaenoptera acutorostrata*) were collected in the offshore area while the remaining species were collected inshore. Details on location and sampling procedures are described elsewhere (Møller *et al.* 2006 in prep.).

### Sample preparation

Prior to extraction, samples were taken from the freezer (-50°C), individual samples were cut and homogenised as appropriate using either a blender (OBH Nordica 6720, Denmark) or meat minzer (Robot Coupe R301, France). Large and massive samples (e.g. walrus heart) were allowed to thaw until semi-frozen. From the homogenised material a sub sample of between 0,5g (fatty tissue) and 7g (lean tissue) was transferred to a thick-walled homogenisation glass, weighed

(0.01g) (AND FX-3000, Japan), covered with tinfoil and stored on ice or in the freezer (-20°C) until extraction could be performed the same day.

### **Lipid extraction**

Total lipid was extracted from "wet tissue" by the method of Folch et al (1957). In brief, extraction was performed using 20-fold chloroform:methanol 2:1 (v/v) to 1.0g of wet tissue. Extraction was performed on ice and assisted by homogenisation using a Polytron PT-2000 with a PTA 20S rod (Kinematica AG., Switzerland). Homogenisation was run in 3 to 5 15sec-cycles interrupted by 45sec of cooling. The extract was filtered through filterpaper (Whatman no. 1) into a measuring cylinder (100-250ml) by suction. The knife and homogenisation glass was rinsed in 2 x 5ml solvent and the filter-glass+filterpaper rinsed with additionally 2 x 5ml solvent. The exact volume (0.5ml) was noted and saltwater (0.73% NaCl) added to reach a final ratio of chloroform:methanol:water 8:4:3 (v/v/v). The saltwater volume was adjusted accordingly to the tissue-specific water content.

Extract and saltwater was carefully mixed and allowed to separate overnight (5°C). The following day, the upper phase was discarded and the lower organic phase transferred to a round-bottomed flask (100-250ml) together with 2 x 2.5ml chloroform:methanol 2:1 (v/v) from rinsing the measuring cylinder. The organic solvent was removed by vacuum distillation at 40°C using a water bath (B-480, Büchi, Switzerland) and a rotary evaporator (R-114, Büchi, Switzerland) connected to a teflon-lined vacuum pump (KNF Laboport, Neuberger, Germany) and any water-residues was removed by adding methanol. The dry flask+lipid was immediately weighed (0.1mg) (AND HA-120M, A&D Company, Japan), lipids were transferred to a 7.0ml volume-calibrated glass tubes using 4 x 1.5ml chloroform:methanol 95:5 (v/v) and stored at -80°C. The lipid-free flask was dried passively over night and weighed the following day to give gravimetric estimates of total lipid.

### **Fatty acid methyl esters**

Total lipid extracts were saponified and methylated to produce fatty acid methylesters using a modified method based on Morrison and Smith (1964) [18]. In general, 7.5mg of lipid extract was transferred to 10ml glass tubes added 375µg internal standard (C23:0 ME, >99%, Nu-Chek Prep. Inc., USA). Organic solvents were removed under N<sub>2</sub> at 40°C, 0.7 ml 0.7N NaOH in methanol and 0.3 ml toluene were added and mixed for 10s, the glass was capped tightly and left for 10min at 90°C in a heating block (QBT2, Grant Instruments, England). After saponification, the samples were cooled in water (5°C), and flipped over once before opening. For methylation, 1ml 20% BF<sub>3</sub>-methanol was added followed by 0.5ml 0.05% hydroquinon in methanol, this was mixed for 10sec and left for 2 min at 90°C in a heating block. The samples were cooled, flipped once before opening and 1ml milliQ-H<sub>2</sub>O was added followed by 1 ml heptane and then

mixed for 20sec before transferring the upper organic (heptane) phase to a 3ml glass tube. An additional 1ml of heptane was added to the lower phase, then mixed for 20sec and the resulting upper phase transferred. The pooled upper phases were dried under N<sub>2</sub> (40°C) and dissolved in 2 ml heptane (ca. 3.75µg/µl) and an aliquot was transferred to a GC vial for fatty acid analysis using gaschromatography.

### Gaschromatography

The fatty acid composition was analysed on HP-6890 (Agilent) fitted with an autosampler and split/splitless injector. Injector and detector temperature was set at 250C and 270C, respectively. Helium was used as carrier gas and operated at constant pressure (33.00 psi) with a nominal initial flow of 2.5ml/min. Hydrogen and air flow at the detector was 30.0 ml/min and 400.0ml/min, respectively, with a constant makeup flow of 25.0 ml/min. In general 1µl of sample was injected with 10:1 split ratio and a split flow of 24.7ml/min and a total flow of 29.1ml/min.

FAMES were separated on a 50m x 0.25mm i.d. (0.25µm film thickness) capillary column (CP Select CB for FAME, Chrompack). The initial temperature was set at 50°C and immediately ramped to 140° at 30C/min and held at this temperature for 2 min, then ramped to 180°C at 2°C/min, where it was held for 8.5min, and finally ramped to 250C at 2.5C/min and held for 10min. An entire run took 71.5min – an equilibration time of 3min between runs was allowed. Data was collected at a rate of 10 Hz and analysed using GC ChemStation software (version 10.01, Agilent Technologies). The condition of the column and GC performance in general was checked daily using the quantitative standard GLC 68A (Nu-Chek Prep, Inc.). For identification an additional number of FAME standards were run in every sequence i.e. every 30-60 samples. Standards used were GLC 85, GLC 463, GLC 566B (Nu-Chek Prep, Inc.), a mixed standard of unusual polyunsaturated fatty acids (Nu-Chek Prep, Inc., Larodan, Sigma, Matreya, Inc.), a mixed standard of branched fatty acids (Nu-Chek Prep, Inc., Larodan, Sigma, Matreya, Inc.), and a fish oil L49 (Salmon sp.) of known composition (Aarhus United, Denmark).

## Results

Muscle/meat and blubber from 29 marine species (4 invertebrates, 13 fish, 5 seabirds, 7 marine mammals) were analysed for lipid content (Table 1) and fatty acid composition (Table 2-6). Unless otherwise indicated, species and data given below refer to muscle tissue, except for blue mussel (*Mytilus edulis*) where soft tissue of the mussel was analysed.

### Lipid content

The over all lipid content (mass%) of muscle and soft tissue ranged between 0.8% (Greenland cod, *Gadus ogac*) and 11.7% (Atlantic

salmon, *Salmo salar*) and thus represented the range within fish (Table 1). For the remaining three taxa, lipid content in muscle tissue ranged between 1.0% (snow crab, *Chionoecetes opilio*) and 1.5% (blue mussel, *Mytilus edulis*) within invertebrates, between 3.3% (common eider, *Somateria mollissima*) and 6.7% (kittywake, *Rissa tridactyla*) within seabirds and between 1.5% (walrus, *Odobenus rosmarus*) and 5.1% (minke whale, *Balaenoptera acutorostrata*) within marine mammals (Table 1). Lipid content of blubber ranged between 72.7% (minke whale) and 91.5% (beluga, *Delphinapterus leucas*) (Table 1).

### Fatty acid composition

A maximum of 85 fatty acids (FA) could be identified ranging from C8 to C24. The range represented saturated FA (SFA, including branched-chained i.e. iso-, anteiso-, phytanic – and pristanic acid), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA, dienes - hexaenes). Based on this array of FA, generally more than 96% (92.7-98.8%) of total fatty acids was identified in 28 of the 29 species investigated. In blue mussel (soft tissue) only 85.3±2.7% could be identified. Plasmalogens have been reported in relative large amounts (38.9 mol% of total moles of glycerophospholipids) in the soft tissue of blue mussel [19]. Though we have identified peaks as potential plasmalogens, positive confirmation was not possible. Fatty acid percental distribution is given as percent of total fatty acids.

### Fatty acid classes

Fatty acid composition including SFA, MUFA, PUFA, omega-6 (n-6), omega-3 (n-3) and n-6/n-3 for muscle/soft tissue is presented in Tables 2, 3, 4 and 5 and for blubber in Table 6. In Table 1, the content of PUFA is given as g/kg fresh weight. Differences in the composition were observed at all levels i.e. within and between species as well as within and between tissues. Data on fatty acids of specific importance to human nutrition are given below.

### SFA

Seabirds showed the highest levels of SFA (28.2-33.3%) with common eider, king eider and little auk all containing more than 30%. In the sea birds, stearic acid constituted between 35 and 50 percent of the total SFA in the sea birds. Remaining species (i.e. invertebrates, fish and marine mammals) all had levels below 28.1%. Among the analyzed dietary components the lowest levels of SFA, were found in marine mammal blubber (11.7-19.1%) together with muscle from snow crab (17.7%), ringed seal (18.8%), deepwater redfish (19.0%) and blue mussel (19.6%).

### MUFA

High levels of MUFA, around 50% or higher, were generally found in marine mammals (muscle and blubber) and deepwater fish (deepwater redfish, golden redfish, Greenland halibut). Among these,

highest levels (above 60%) were observed in Greenland halibut (61.0%) and in blubber of beluga (68.2%) and narwhal (70.3%).

## PUFA

The studied marine sources had generally PUFA-levels above 20%, with exception of hooded seal and species with extremely high MUFA-levels (i.e. Greenland halibut, narwhal and beluga). Higher levels of PUFA (above 30%), were identified in harp seal blubber (30.1%), walrus muscle (30.2%), king eider (32.4%), fish (excl. Greenland halibut) (30.0-57.9%) and invertebrates (44.2-59.3%). Highest total PUFA levels occurred in invertebrates and lean fish such as haddock (54.1%), snow crab (56.4%) Atlantic cod (57.8%), Greenland cod (57.9%) and Iceland scallop (59.3%). Considering the mass PUFA per kg wet weight, blubber from harp seal and ringed seal were the best dietary sources. Harp seal contained 271 g/kg and ringed seal 262 g/kg, which was considerably higher than in any of the other tissues analyzed (Table 1). In the other taxa, the highest PUFA mass was found in the species with the highest lipid content. Thus, among the seabirds, kittiwake and little auk were the richest sources, containing 14.5 and 13.5 g/kg, respectively. Among the fish Arctic char and Atlantic salmon had the highest PUFA content, with 36.5 and 33.6 g/kg. However, the mass of PUFA was very similar among the invertebrates studied, ranging between 5.2 and 6.8 g/kg, with blue mussel having the highest content and Northern shrimp the lowest.

## n-6 PUFA and n-3 PUFA

The highest n-6 PUFA levels occurred in harp seal (muscle: 5.6%), spottet wolffish (6.2%), blue mussel (7.2%), walrus (7.6%) and seabirds (6.1-14.7%). Exceptional high percentage of n-6 PUFAs (i.e. >10%) were found in king eider (12.4%) and common eider (14.7%). On a mass basis, blubber from harp seal and hooded seal were the best sources for n-6 fatty acids, both species had about 21 g n-6 PUFA per kg blubber (Table 1). It is also notable that, despite relatively large variation in total lipid content, the mass of n-6 PUFA was very similar in four out of five bird species, ranging from 4.1 to 4.8 g/kg.

Levels of n-3 PUFA above 20% were identified in Brünnichs guillemot (20.8%), walrus (muscle: 21.7%; blubber: 23.3%), harp seal (muscle: 21.8%; blubber: 26.8%), ringed seal (blubber: 26.8%), fish (excl. Greenland halibut) (26.7-54.3%) and invertebrates (37.8-54.6%). Levels above 30% were, apart from invertebrates, also found in lean fish (34.7-54.3%), in capelin (32.4%) and Arctic char (33.8%). Very high levels of n-3 PUFAs (about 50%) correlated with low lipid content (<1.1 mass%) and were found in haddock (49.8%), snow crab (51.3%), Greenland cod (53.8%), Atlantic cod (54.3%) and Iceland scallop (54.6%). Since n-3 PUFA was the dominating PUFA in almost all taxa, the mass of n-3 PUFA was similar to the mass of total PUFA, described above (Table 1).

The ratio between n-6 and n-3 PUFAs ranged from 0.06 to 1.06 in muscle/soft tissue and from 0.08 to 0.17 in blubber. Even though n-

6/n-3 in muscle of capelin (*Mallotus villosus*) and common eider differed by a factor 20, all samples analysed had a low n-6/n-3 ratio (i.e. <1.1). The ratio in invertebrates (0.07-0.19), fish (0.06-0.18) and marine mammal blubber (0.08-0.17) was very low, somewhat higher in marine mammal muscle (0.15-0.37) and highest in the seabirds (0.35-1.06).

### **Essential fatty acid (EFA)**

The EFA linoleic acid (LA, 18:2n-6) ranged between 0.6% and 3.7% in muscle/soft tissue and between 0.9% and 1.6% in blubber. All seabirds, all marine mammals (except narwhal blubber and walrus muscle and blubber), 6 out of 11 fish and 2 out of 4 invertebrates all had LA levels above 1.0%. Among these the best sources of LA (>2.0%) were seabirds (2.1-3.7%), harp seal (2.6%) and blue mussel (2.3%).

The other EFA,  $\alpha$ -linolenic acid (ALA, 18:3n-3) occurred at 0.06% to 1.2% in muscle/soft tissue and between 0.2% and 0.8% in blubber (Table 7). All seabirds contained more than 0.5% ALA. Among the other taxa, blue mussel and snow crab (1.2% and 0.8%, respectively), Arctic char and Atlantic salmon (0.9% and 0.7%, respectively) and blubber from minke whale and harp seal (0.7% and 0.8%), also had ALA percentages higher than 0.5%.

### **Long-chained polyunsaturated fatty acids**

The percentage of eicosanopentaenoic acid (EPA, 20:5n-3) ranged between 4.9% and 32.3% in muscle/soft tissue and from 3.5% to 9.0% in blubber. Levels above 10% EPA were found in all invertebrates (19.4-32.3%), seven lean fish species (14.6-17.8%), capelin (13.9%) and Arctic char (10.1%). Snow crab represented the highest EPA concentration recorded (32.3%). Seal blubber was the best source of EPA, the best being ringed seal blubber containing 80.6 g/kg, followed by harp seal blubber with 65.3 g/kg. Within fish, fatty fish were good sources, especially Arctic char and Atlantic salmon containing 9.1 and 9.3 g/kg, respectively. Among seabirds little auk and kittiwake were the best sources, containing 45.7 and 47.3 g/kg respectively. Lean fish and invertebrates contained between 1.2 and 3.2 g/kg (Table 1).

Levels above 10% DHA were found in harp seal blubber (10.6%), all invertebrates (11.6-23.5%) and all fish species, except Greenland halibut (13.0-34.4%). The highest levels (above 30% DHA) were found in the leanest fish species such as Greenland cod, Atlantic cod and haddock. However, looking at the mass fatty acids per gram tissue, seal blubber is also the best source of DHA, containing between 62.7 (hooded seal) and 95.2 (harp seal). The fish with the highest lipid content were also excellent sources, Arctic char and Atlantic salmon contained 12.4 and 15.2 g/kg, respectively.

Overall arachidonic acid (AA, 20:4n-6) ranged from 0.4% to 10.5% in muscle/soft tissue and between 0.2% and 0.7% in blubber.



Levels at 3% or higher, were found in Atlantic wolffish (3.0%), blue mussel (3.3%), spotted wolffish (4.7%), walrus (muscle: 6.1%) and seabirds (excl. kittywake) (3.5-10.5%). The highest AA levels (above 6.5%) were identified in king eider (9.1%) and common eider (10.5%). The highest content of AA per kg tissue was found in walrus blubber, containing 5.6 g/kg. Seabird muscle was the only tissue, beside blubber, that contained more than 1 g/kg AA. Common eider and king eider were the best sources among the seabirds, containing 3.4 and 3.0 g/kg respectively.

### **Nutritionally relevant branched-chain fatty acids**

Pristanic acid (PRI, 2,6,10,14-tetramethylpentadecanoic acid) was generally found in low concentrations, ranging from non-detectable in harp seal and ringed seal blubber as well as in Greenland cod to 0.33% in muscle of narwhal (*Monodon monoceros*).

Phytanic acid (PHY, 3,7,11,15-tetramethylhexadecanoic acid) on the other hand was detected in all species and tissues ranging from 0.11% in muscle of Northern shrimp (*Pandalus borealis*) to 1.1% in muscle of little auk (*Alle alle*). Levels were generally low in invertebrates (<0.23%). Apart from little auk, high concentrations were found in capelin (1.0%), spotted wolffish (0.9%) and American plaice (0.8%). The richest dietary sources of the phytanic and pristanic acid was hooded seal and beluga blubber, containing 5.0 and 4.7 g/kg, respectively. Good sources among the other species were Greenland halibut with 0.7 g per kg wet weight and little auk with 0.6 g/kg. Since pristanic acid and phytanic acid have similar effects [20], we have calculated the total intake of these two phytol metabolites per kg consumed tissue. Hooded seal and beluga blubber contained the highest amount of phytanic and pristanic acid, 5.0 and 4.7 g/kg, respectively. It is noteworthy that there is a large variation in the content of these multibranched fatty acids within the different taxa. Thus seabirds contained between 0.1 to 0.6 g/kg, with little auk being the best source, and fish contained between 0.0 and 0.7 g/kg, with Greenland halibut having the highest concentration.

## **Discussion**

The population of Greenland has recently gone through a rapid change in diet, moving away from a traditional marine diet to a more western-like diet, based on imported foodstuffs. This dietary transition is mainly driven by the general socio-cultural changes linked to a more western-like life-style, but awareness of contamination of the diet may also have had an effect. As a consequence, a reduction in the intake of n-3 PUFAs and increased intake of saturated and n-6 PUFAs, combined with the more western-like sedentary life-style, have raised new concerns of an increased incidence of metabolic syndrome and other life-style related diseases.

In the present study, we have identified marine resources that, as components of the traditional diet in Greenland, have a high dietary

lipid quality (e.g. rich in n-3 PUFAs, low in saturated fatty acids that raise the LDL/HDL ratio) without being enriched in contaminants. Opening the possibility of including the lipid quality of the traditional foodstuffs in the dietary recommendations of the Greenlandic population.

### **Traditional diet and international dietary recommendations**

Based on Nordic nutrition recommendations (2004) lipids in the diet should represent no more than 30% of total energy intake (E%) [21]. The intake of SFA including trans-FA should be limited to 10 E%, MUFA should make up 10-15 E% and PUFA 5-10 E% including about 1 E% of n-3 FA. Hence the relative contribution in dietary lipids is currently recommended at a ratio of 30:50:20 (SFA:MUFA:PUFA). Although the content of saturated fatty acids is above 10 % in all studied tissues, it should be noted that stearic acid constitutes a large portion of these fatty acids in sea birds and in mammalian muscles. Since it is established that stearic acid do not raise the LDL-HDL ratio [22], the SFA obtained from these tissues will have a less adverse effects on plasma lipoprotein profile, than expected from the total SFA level.

In 1974 the composition of dietary FA in the Greenland diet was within dietary recommendations, but as a result of the recent changes in dietary habits this is no longer is the case. In a recent dietary study by Deutch *et al.* an increase in lipid to 40 E% was mainly due to an increase in SFA and resulted in a ratio of about 40:45:15 (SFA:MUFA:PUFA) [4]. Hence, in the present Greenlandic diet, the intake of SFA is generally above recommendations, while MUFA and PUFA intake is below the recommendations. As shown in tables 2-6, most dietary components investigated in the present study have a fatty acid composition that will adjust the dietary intake toward the recommendations. Based on an assumption of 70% tissue water-content, components with less than 5.4 mass% lipid represent a lipid energy-percent of less than 40%, hence contributing to a reduction in lipid E% intake compared to the reported present intake [4]. The only dietary components that would not do this are Atlantic salmon, Greenland halibut, Arctic char, kittiwake and marine mammal blubber (Table 1). A few other components are above the recommended 30 E% lipid (i.e. >3.7 mass% lipid), namely muscle from minke whale, little auk and capelin (Table 1).

Recommended daily intake of linolic acid is 14-17g (Institute of Medicine 2002) contributing to a total of 4-8 E% of n-6 PUFA. For n-3 PUFA 2g  $\alpha$ -linolenic acid and 0.2g of long-chained n-3 PUFA (EPA, DHA) is recommended as minimum daily intake (European Commission Directorate 2001). None of the components of the marine traditional diet fulfils the requirements for n-6 PUFA, which emphasizes the importance of combining the traditional diet with high quality products of plant origin that can supply the n-6 PUFA, while a weekly intake of 60 g harp seal blubber is enough to alone cover the minimum requirement of the n-3 PUFAs.

In the Greenland population, the body mass index (BMI) has increased significantly in both men and women and as a result, the number of obese men (53%) and women (13%) has increased along with a significant increase in plasma TAG and cholesterol (Deutch *et al.* 2006a submitted). This dramatic increase in obesity is followed by an increasing prevalence of non-insulin dependent diabetes mellitus (i.e. type 2-diabetes) [23]. A similar rapid increase in prevalence in components of the metabolic syndrome have also been observed in other Arctic populations going through a rapid shift away from the traditional marine diets e.g. Alaskan Yup'ik Inuit[24]. In this population the plasma content of n-3 PUFAs was strongly correlated with improved glucose tolerance, decreased fasting insulin and plasma HDL concentration, while it was negatively correlated to plasma triglyceride and body weight [15, 24]. Thus the shift from the marine diet increases the risk of developing metabolic syndrome, and a combination of a more sedentary life-style with decreased intake of marine lipids, further enhances this risk. In this context, it is important to stress that several components of the marine lipids, potentially could prevent development of several of the components of the metabolic syndrome. Thus, highly unsaturated fatty acids of the n-3 type have been found to lead to a repartitioning of hepatic fatty acid away from TAG synthesis and towards fatty acid oxidation and/or thermogenesis, due to their activation of the peroxisome proliferation activator receptor- $\alpha$  (PPAR- $\alpha$ ) and inhibition of the sterol regulating element binding protein -1 [25, 26]. However, several recent studies have shown that phytanic and pristanic acid have similar effects, being efficient activators of PPAR- $\alpha$  and possibly also PPAR- $\gamma$  [20, 27]., Intake of either these fatty acids, or the metabolic precursor, phytol, causes induction of several enzymes in the  $\beta$ -oxidation of fatty acid in both peroxisomes and mitochondria, as well as increases the expression of uncoupler protein-1 and inducing differentiation of brown adipose tissue [28, 29]. The former effects are expected to reduce the lipid content in peripheral tissue as well as the circulating free fatty acid level, and the latter leads to increased energy expenditure through thermogenesis, which would further increase fatty acid  $\beta$ -oxidation. Phytanic acid, in contrast to other fatty acids, also specifically up-regulates glucose uptake in hepatocytes at physiological concentrations [30]. Since lipid deposition in liver and skeletal muscles is a major risk factor for developing insulin resistance [31] and hypoglycaemia is a major risk factor for the transduction of insulin resistance to type-II diabetes, these effects are expected to decrease the risk of developing both metabolic syndrome and type-II diabetes. It is noteworthy that phytanic and pristanic acid have these effects, at concentrations equal to the concentration in human plasma [27]. Therefore dietary components rich in phytanic acid, such as blubber, Greenland halibut and little auk, could potentially be used as natural sources of PPAR-agonists, in dietary regimes specifically aiming at decreasing the risk of developing metabolic syndrome. However, it must also be recognized that subjects with certain uncommon genetic defects in peroxisomal lipid metabolism, such as Refsums disease, should avoid foodstuffs containing high concentrations of branched-chain fatty acids [32]. Thus, the presented data on phytanic and pris-

anic acid content in the traditional foodstuffs, could also be used in selecting components of the traditional diets with lowest possible content of these fatty acids, such as invertebrates, lean fish and mammalian muscle, for persons in Greenland suffering from these diseases.

### **Advice based on LQC vs. contaminants**

Based on our results we have identified a number of good sources for each of the lipid quality components with a top 3 listed in Table 7. However dietary advice should not be based on this information alone but be balanced with other nutrients and contaminants. To illustrate the effect of combining lipid data with contaminant data we have estimated the relative intake of LQC as compared to contaminants using Hg and PCB concentrations (mg/kg ww)[16] normalized for total lipid (mass%) (Table 7). Hence, we obtain a parameter describing the intake of contaminant per mass fatty acid consumed.

When LQCs and contaminant corrected LQCs (LQC/Hg and LQC/PCB) were compared, no instances occurred where the same resource was appointed as the best source for all three measures. This was in accordance to our expectations, since mussel generally contain high levels of Hg contrasting blubber which contain high levels of PCB [16]. Only twice was the best LQC source picked as one of the best contaminant-corrected sources. This was so for DHA and Atlantic cod (LQC/PCB) and for SFA and ringed seal blubber (LQC/Hg). Only in 9 out of 58 other possible instances was a top 3 LQC component picked as a contaminant-corrected top 3 component (Table 7).

By balancing lipid quality and contaminant exposure we have been able to identify ringed seal blubber as the best source for 9 out of 10 PCB-corrected LQCs. Only for pristanic acid+phytanic acid (PRI+PHY) was harp seal blubber picked as best source with ringed seal blubber as second best (Table 7). For the Hg-corrected LQCs capelin was identified as the overall best source. It was within the top 3 best sources for 9 out of 10 components and assigned as the best sources in 6 of these (i.e. PRI+PHY, EPA, SFA, MUFA, PUFA and n-3 PUFA). Arctic char, king eider and Atlantic cod were best Hg-corrected sources for LA and LNA, AA and DHA, respectively.

Based on this, we suggest that contaminant corrected nutritional parameters are applied in future dietary models. The nutritional parameters should also be graded relative to their health risk/benefits in relation to public health. In addition this model should include specific information on dietary recommendations related to population groups and diseases.

The fatty acid data generated in this study emphasize the high lipid quality of marine resources and their health implications as part of the traditional diet of West Greenland. Despite a pronounced difference in fatty acid signatures and balance between FA classes, all components represent a favorable balance between n-6 PUFA and n-3 PUFA (1 or less), and apart from blubber, Atlantic salmon, Arctic char

and Greenland halibut, all are categorized as lean foodstuffs. Despite the high fat content in blubber, harp and ringed seal blubber is an excellent source of the n-3 PUFA, but blubber unfortunately also has high concentrations of persistent organic contaminants. However, a minor intake of these blubber types could be used to obtain a substantial increase in the intake of these health-promoting fatty acids, without increasing the contaminant-exposure significantly. The differences in the balance between FA and FA classes (SFA:MUFA:PUFA) should be considered advantageous as it allows for maneuverability when facing a number of different dietary scenarios. Hence, our data may be used to advice about diet in Arctic societies, aiming at the part of the population where diet and lifestyle imply a high risk of developing metabolic syndrome. Particularly under these conditions our results on the lipid quality of marine resources can be of assistance in giving targeted dietary advice, taking both the positive effects and the contaminant content into consideration.

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**Table 1.**

Lipid content (mean  $\pm$  stdev) and content of PUFA and phytanic acid+pristanic acid (Pri+Phy) as mg/kg ww. (mean) of marine key species and tissues (muscle, soft tissue, blubber) in the traditional diet of West Greenland. Species are grouped according to taxa and with in taxa listed according to ascending lipid content.

Taxonomic group - Common name	Scientific name	Tissue	n	Lipid content (% ww)	Range (Min - Max)	Total PUFA g/kg	n-6 g/kg	n-3 g/kg	Phy+Pri g/kg
<b>Invertebrates</b>									
Snow crab	<i>Chionoecetes opilio</i>	muscle	10	1,0 $\pm$ 0,2	0,6 - 1,4	5.5	0.4	5.0	0.0
Islandic scallop	<i>Chlamys islandica</i>	muscle	10	1,0 $\pm$ 0,1	0,8 - 1,1	5.9	0.4	5.5	0.0
Northern shrimp	<i>Pandalus borealis</i>	muscle	10	1,2 $\pm$ 0,2	1,0 - 1,5	5.2	0.3	4.8	0.0
Blue mussel	<i>Mytilus edulis</i>	soft tissue	10	1,5 $\pm$ 0,3	1,1 - 2,0	6.8	1.0	5.5	0.1
<b>Fish</b>									
Greenland cod	<i>Gadus ogac</i>	muscle	10	0,8 $\pm$ 0,1	0,6 - 0,9	4.5	0.3	4.1	0.0
Atlantic cod	<i>Gadus morhua</i>	muscle	10	0,9 $\pm$ 0,0	0,8 - 0,9	5.1	0.3	4.8	0.1
Haddock	<i>Melanogrammus aeglefinus</i>	muscle	10	0,9 $\pm$ 0,1	0,8 - 1,1	5.0	0.3	4.6	0.1
Short-horned sculpin	<i>Myoxocephalus scorpius</i>	muscle	10	1,1 $\pm$ 0,3	0,8 - 1,5	5.1	0.4	4.7	0.1
American plaice	<i>Hippoglossoides platessoides</i>	muscle	10	1,3 $\pm$ 0,7	0,7 - 2,9	5.7	0.4	5.1	0.1
Atlantic wolffish	<i>Anarhichas lupus</i>	muscle	10	1,3 $\pm$ 0,5	0,8 - 2,4	6.1	0.6	5.5	0.1
Spotted wolffish	<i>Anarhichas minor</i>	muscle	10	1,6 $\pm$ 1,1	0,7 - 4,4	6.9	1.0	5.6	0.1
Deepwater redfish	<i>Sebastes mentella</i>	muscle	10	2,4 $\pm$ 1,2	0,9 - 4,6	7.0	0.7	6.3	0.1
Golden redfish	<i>Sebastes marinus</i>	muscle	10	2,9 $\pm$ 1,8	1,5 - 6,9	8.9	0.8	8.0	0.2
Capelin	<i>Mallotus villosus</i>	muscle	10	3,8 $\pm$ 1,4	2,2 - 5,8	13.4	0.7	12.4	0.4
Greenland halibut	<i>Reinhardtius hippoglossoides</i>	muscle	10	8,7 $\pm$ 2,4	3,0 - 11,6	12.7	1.3	10.7	0.7
Arctic char	<i>Salvenius alpinus</i>	muscle	10	9,0 $\pm$ 2,1	5,7 - 12,2	33.6	2.5	30.4	0.4
Atlantic salmon	<i>Salmo salar</i>	muscle	10	11,7 $\pm$ 2,4	6,9 - 14,2	36.5	2.7	33.1	0.4
<b>Seabirds</b>									
Common eider	<i>Somateria mollissima</i>	muscle	10	3,3 $\pm$ 0,5	2,6 - 4,1	9.5	4.8	4.6	0.1
Brünnichs guillemot	<i>Uria lomvia</i>	muscle	10	3,3 $\pm$ 0,5	2,3 - 4,4	9.3	4.2	6.8	0.2
King eider	<i>Somateria spectabilis</i>	muscle	10	3,3 $\pm$ 0,6	2,5 - 4,5	10.8	4.2	6.4	0.3
Little auk	<i>Alle alle</i>	muscle	10	4,9 $\pm$ 0,6	3,6 - 5,9	13.5	4.3	9.0	0.6
Kittiwake	<i>Rissa tridactyla</i>	muscle	10	6,7 $\pm$ 0,9	5,4 - 8,0	14.5	4.1	10.2	0.3
<b>Marine mammals</b>									
Walrus	<i>Odobenus rosmarus</i>	muscle	10	1,1 $\pm$ 0,3	0,8 - 1,5	3.4	0.9	2.5	0.0
		blubber	10	80,7 $\pm$ 3,9	72,8 - 85,6	214.8	19.8	188.3	1.8
Narwhal	<i>Monodon monoceros</i>	muscle	7	1,3 $\pm$ 0,3	1,0 - 1,9	2.4	0.5	1.9	0.1
		blubber	7	88,8 $\pm$ 4,0	83,1 - 93,4	106.7	14.2	85.7	3.7
Beluga	<i>Delphinapterus leucas</i>	muscle	10	1,7 $\pm$ 0,5	0,9 - 2,5	3.5	0.6	2.8	0.1
		blubber	10	91,5 $\pm$ 2,6	88,2 - 96,0	125.1	16.3	101.5	4.7
Harp seal	<i>Pagophilus groenlandicus</i>	muscle	10	2,2 $\pm$ 0,9	1,2 - 4,2	6.0	1.3	4.7	0.1
		blubber	10	90,2 $\pm$ 2,8	87,1 - 95,3	271.8	21.2	241.9	3.2
Hooded seal	<i>Cystophora cristata</i>	muscle	9	2,0 $\pm$ 0,7	1,3 - 3,3	3.7	1.0	2.6	0.2
		blubber	9	88,3 $\pm$ 4,2	78,7 - 92,0	188.4	21.4	160.8	5.0
Ringed seal	<i>Phoca hispida</i>	muscle	10	3,1 $\pm$ 1,0	2,1 - 5,3	6.9	1.1	5.6	0.1
		blubber	10	89,6 $\pm$ 2,9	84,2 - 92,7	262.5	17.4	235.7	1.7
Minke whale	<i>Balaenoptera acutorostrata</i>	muscle	4	5,1 $\pm$ 3,3	1,5 - 8,4	11.4	1.4	9.7	0.3
		blubber	3	72,7 $\pm$ 5,9	65,8 - 76,7	162.6	16.3	141.2	4.4



**Table 2.**

Fatty acid composition of total lipid of invertebrates (muscle, soft tissue). Individual fatty acids are given as mass% (mean  $\pm$  stdev) of total fatty acids.

<b>Invertebrates</b>				
Fatty acid	Snow crab	Iceland scallop	N. shrimp	Blue mussel
14:0	0,24 $\pm$ 0,03	1,81 $\pm$ 0,20	1,88 $\pm$ 0,19	2,29 $\pm$ 0,44
Pristanic acid	0,03 $\pm$ 0,02	0,05 $\pm$	0,02 $\pm$ 0,00	0,12 $\pm$ 0,03
16:0	12,18 $\pm$ 0,74	15,04 $\pm$ 0,51	17,72 $\pm$ 1,27	12,42 $\pm$ 0,85
Phytanic acid	0,21 $\pm$ 0,05	0,15 $\pm$ 0,07	0,11 $\pm$ 0,04	0,23 $\pm$ 0,07
17:0	0,66 $\pm$ 0,07	0,50 $\pm$ 0,05	0,34 $\pm$ 0,05	0,53 $\pm$ 0,07
ai-17:0	0,48 $\pm$ 0,16	0,29 $\pm$ 0,03	0,11 $\pm$ 0,03	0,17 $\pm$ 0,02
18:0	2,55 $\pm$ 0,72	5,30 $\pm$ 0,36	1,74 $\pm$ 0,11	2,61 $\pm$ 0,30
iso-18:0	0,53 $\pm$ 0,10	0,05 $\pm$	0,19 $\pm$ 0,03	0,16 $\pm$ 0,02
14:1n-5	0,06 $\pm$ 0,01	0,03 $\pm$ 0,00	0,10 $\pm$ 0,02	0,13 $\pm$ 0,01
16:1n-7	2,95 $\pm$ 0,45	1,63 $\pm$ 0,13	4,38 $\pm$ 0,43	5,41 $\pm$ 0,85
16:1n-9	0,12 $\pm$ 0,02	0,17 $\pm$ 0,02	0,14 $\pm$ 0,01	0,16 $\pm$ 0,03
16:1n-11	0,19 $\pm$ 0,03	0,18 $\pm$ 0,02	0,15 $\pm$ 0,02	0,14 $\pm$ 0,03
18:1n-5	0,65 $\pm$ 0,15	0,21 $\pm$ 0,02	0,47 $\pm$ 0,07	0,18 $\pm$ 0,02
18:1n-7	7,63 $\pm$ 0,25	3,40 $\pm$ 0,39	6,17 $\pm$ 0,75	2,42 $\pm$ 0,16
18:1n-9	8,24 $\pm$ 0,52	1,96 $\pm$ 0,16	14,32 $\pm$ 1,02	3,29 $\pm$ 0,59
18:1n-11	0,15 $\pm$ 0,01	0,07 $\pm$ 0,01	0,19 $\pm$ 0,06	0,08 $\pm$ 0,02
18:1n-13 <sup>a</sup>	0,19 $\pm$ 0,13	0,17 $\pm$ 0,06	0,13 $\pm$ 0,03	0,57 $\pm$ 0,12
20:1n-7	0,62 $\pm$ 0,09	1,26 $\pm$ 0,32	0,51 $\pm$ 0,13	1,25 $\pm$ 0,41
20:1n-9	0,49 $\pm$ 0,08	1,04 $\pm$ 0,06	1,50 $\pm$ 0,29	3,88 $\pm$ 0,52
20:1n-11	0,30 $\pm$ 0,05	0,71 $\pm$ 0,07	0,23 $\pm$ 0,08	0,75 $\pm$ 0,13
22:1n-9	0,04 $\pm$	$\pm$	0,28 $\pm$ 0,14	$\pm$
22:1n-11	0,05 $\pm$ 0,02	$\pm$	0,71 $\pm$ 0,22	$\pm$
24:1n-9	0,01 $\pm$ 0,00	0,02 $\pm$	0,29 $\pm$ 0,08	0,02 $\pm$ 0,00
16:2n-4	0,02 $\pm$ 0,01	0,07 $\pm$ 0,02	0,09 $\pm$ 0,01	0,44 $\pm$ 0,08
16:3n-3	0,10 $\pm$ 0,01	0,22 $\pm$ 0,08	0,02 $\pm$ 0,01	0,13 $\pm$ 0,04
16:3n-4	0,23 $\pm$	0,24 $\pm$ 0,32	0,11 $\pm$ 0,02	0,68 $\pm$ 0,17
18:2n-4	0,24 $\pm$ 0,03	0,44 $\pm$ 0,07	0,16 $\pm$ 0,01	0,21 $\pm$ 0,04
18:2n-6, LA	0,53 $\pm$ 0,07	0,73 $\pm$ 0,08	1,11 $\pm$ 0,09	2,37 $\pm$ 0,56
18:3n-3, ALA	0,70 $\pm$ 0,30	0,33 $\pm$ 0,10	0,06 $\pm$ 0,01	1,23 $\pm$ 0,25
18:3n-6	0,23 $\pm$ 0,02	0,18 $\pm$ 0,01	0,11 $\pm$ 0,01	0,25 $\pm$ 0,02
18:4n-3 <sup>b</sup>	0,19 $\pm$ 0,05	4,24 $\pm$ 0,81	0,38 $\pm$ 0,10	2,70 $\pm$ 0,71
20:2n-6	0,41 $\pm$ 0,08	0,66 $\pm$ 0,06	0,17 $\pm$ 0,02	0,71 $\pm$ 0,08
20:3n-3	0,06 $\pm$ 0,01	0,15 $\pm$ 0,03	0,04 $\pm$ 0,01	0,07 $\pm$ 0,01
20:3n-6	0,07 $\pm$ 0,03	0,12 $\pm$ 0,01	0,06 $\pm$ 0,01	0,22 $\pm$ 0,03
20:4n-3 <sup>c</sup>	0,22 $\pm$ 0,07	0,28 $\pm$ 0,05	0,25 $\pm$ 0,02	0,39 $\pm$ 0,09
20:4n-6, AA	2,87 $\pm$ 0,64	1,96 $\pm$ 0,24	1,18 $\pm$ 0,28	3,34 $\pm$ 0,76
20:5n-3, EPA	32,34 $\pm$ 1,56	23,41 $\pm$ 0,99	22,05 $\pm$ 1,08	19,44 $\pm$ 1,34
21:5n-3	0,74 $\pm$ 0,09	1,39 $\pm$ 0,07	0,32 $\pm$ 0,05	0,62 $\pm$ 0,09
22:3n-3 <sup>d</sup>	0,25 $\pm$ 0,07	0,13 $\pm$ 0,02	0,05 $\pm$ 0,03	0,22 $\pm$ 0,04
22:5n-3	2,16 $\pm$ 0,36	0,86 $\pm$ 0,09	0,74 $\pm$ 0,22	1,17 $\pm$ 0,19
22:5n-6	0,40 $\pm$ 0,08	0,32 $\pm$ 0,03	0,09 $\pm$ 0,02	0,25 $\pm$ 0,02
22:6n-3, DHA	14,43 $\pm$ 0,48	23,51 $\pm$ 1,44	17,02 $\pm$ 0,62	11,59 $\pm$ 0,55
Identified%	96,85 $\pm$ 0,65	95,13 $\pm$ 0,86	97,25 $\pm$ 0,25	85,30 $\pm$ 2,72
SAFA	17,67 $\pm$ 1,03	24,06 $\pm$ 0,41	23,08 $\pm$ 1,16	19,63 $\pm$ 0,98
MUFA	22,74 $\pm$ 0,68	11,81 $\pm$ 0,58	29,93 $\pm$ 1,12	19,23 $\pm$ 0,86
PUFA	56,43 $\pm$ 1,34	59,26 $\pm$ 0,92	44,24 $\pm$ 1,26	46,44 $\pm$ 1,59
n-6	4,55 $\pm$ 0,80	3,96 $\pm$ 0,23	2,72 $\pm$ 0,23	7,18 $\pm$ 0,47
n-3	51,29 $\pm$ 0,94	54,63 $\pm$ 1,13	41,11 $\pm$ 1,29	37,80 $\pm$ 1,76
n-6/n-3	0,09 $\pm$ 0,02	0,07 $\pm$ 0,01	0,07 $\pm$ 0,01	0,19 $\pm$ 0,02

**Table 3.**Fatty acid composition of total lipid of fish (muscle). Individual fatty acids are given as mass% (mean  $\pm$  stdev) of total fatty acids.

Fish	Greenland cod	Atlantic cod	Haddock	S. sculpin	American plaice	Atlantic wolffish	Spotted wolffish
Fatty acid							
14:0	0,84 $\pm$ 0,26	0,77 $\pm$ 0,11	0,81 $\pm$ 0,11	1,90 $\pm$ 0,57	2,41 $\pm$ 0,79	1,34 $\pm$ 0,70	1,90 $\pm$ 0,82
Pristanic acid	$\pm$	0,02 $\pm$ 0,01	0,05 $\pm$ 0,04	0,02 $\pm$ 0,01	0,07 $\pm$ 0,03	0,03 $\pm$ 0,01	0,03 $\pm$ 0,01
16:0	17,72 $\pm$ 0,52	17,38 $\pm$ 0,98	17,80 $\pm$ 1,04	14,74 $\pm$ 0,97	16,17 $\pm$ 1,57	13,60 $\pm$ 1,08	11,89 $\pm$ 1,58
Phytanic acid	0,52 $\pm$ 0,15	0,56 $\pm$ 0,07	0,64 $\pm$ 0,11	0,48 $\pm$ 0,04	0,78 $\pm$ 0,16	0,52 $\pm$ 0,15	0,85 $\pm$ 0,20
17:0	0,18 $\pm$ 0,06	0,18 $\pm$ 0,04	0,31 $\pm$ 0,07	0,19 $\pm$ 0,02	0,20 $\pm$ 0,06	0,21 $\pm$ 0,07	0,41 $\pm$ 0,14
ai-17:0	0,07 $\pm$ 0,02	0,08 $\pm$ 0,04	0,13 $\pm$ 0,06	0,20 $\pm$ 0,05	0,12 $\pm$ 0,05	0,17 $\pm$ 0,11	0,24 $\pm$ 0,09
18:0	3,87 $\pm$ 0,24	3,87 $\pm$ 0,33	3,83 $\pm$ 0,23	3,82 $\pm$ 0,40	3,52 $\pm$ 0,69	4,03 $\pm$ 0,71	3,95 $\pm$ 0,59
iso-18:0	0,11 $\pm$ 0,03	0,12 $\pm$ 0,03	0,16 $\pm$ 0,03	0,19 $\pm$ 0,03	0,15 $\pm$ 0,04	0,19 $\pm$ 0,06	0,24 $\pm$ 0,04
14:1n-5	0,04 $\pm$ 0,01	0,06 $\pm$ 0,02	0,08 $\pm$ 0,01	0,18 $\pm$ 0,03	0,20 $\pm$ 0,06	0,13 $\pm$ 0,07	0,33 $\pm$ 0,17
16:1n-7	1,35 $\pm$ 0,22	1,36 $\pm$ 0,25	1,64 $\pm$ 0,22	7,01 $\pm$ 1,87	4,27 $\pm$ 1,95	4,56 $\pm$ 1,62	4,03 $\pm$ 1,76
16:1n-9	0,34 $\pm$ 0,03	0,26 $\pm$ 0,05	0,20 $\pm$ 0,03	0,41 $\pm$ 0,07	0,25 $\pm$ 0,04	0,43 $\pm$ 0,08	0,48 $\pm$ 0,13
16:1n-11	0,21 $\pm$ 0,04	0,19 $\pm$ 0,02	0,27 $\pm$ 0,06	0,34 $\pm$ 0,12	0,35 $\pm$ 0,08	0,24 $\pm$ 0,06	0,43 $\pm$ 0,12
18:1n-5	0,24 $\pm$ 0,03	0,25 $\pm$ 0,05	0,26 $\pm$ 0,04	0,30 $\pm$ 0,07	0,26 $\pm$ 0,09	0,32 $\pm$ 0,04	0,47 $\pm$ 0,08
18:1n-7	4,22 $\pm$ 0,84	4,09 $\pm$ 0,60	4,64 $\pm$ 0,38	4,67 $\pm$ 0,67	4,45 $\pm$ 0,72	5,71 $\pm$ 1,43	5,63 $\pm$ 1,06
18:1n-9	6,89 $\pm$ 0,79	6,70 $\pm$ 0,50	6,48 $\pm$ 0,61	9,15 $\pm$ 0,77	9,01 $\pm$ 2,53	12,04 $\pm$ 1,93	10,52 $\pm$ 3,17
18:1n-11	0,77 $\pm$ 0,24	0,79 $\pm$ 0,16	0,52 $\pm$ 0,11	0,80 $\pm$ 0,56	0,60 $\pm$ 0,29	0,67 $\pm$ 0,43	0,76 $\pm$ 0,39
18:1n-13 <sup>a</sup>	0,08 $\pm$ 0,03	0,08 $\pm$ 0,02	0,19 $\pm$ 0,11	0,55 $\pm$ 0,24	0,28 $\pm$ 0,15	0,20 $\pm$ 0,12	0,40 $\pm$
20:1n-7	0,14 $\pm$ 0,06	0,20 $\pm$ 0,15	0,28 $\pm$ 0,07	0,56 $\pm$ 0,16	0,59 $\pm$ 0,26	0,51 $\pm$ 0,25	0,95 $\pm$ 0,28
20:1n-9	1,45 $\pm$ 0,49	1,77 $\pm$ 0,39	1,29 $\pm$ 0,17	2,43 $\pm$ 1,30	3,77 $\pm$ 2,00	2,50 $\pm$ 1,92	2,48 $\pm$ 1,51
20:1n-11	0,25 $\pm$ 0,04	0,33 $\pm$ 0,06	0,46 $\pm$ 0,24	0,42 $\pm$ 0,23	0,77 $\pm$ 0,28	0,60 $\pm$ 0,31	2,89 $\pm$ 1,39
22:1n-9	0,08 $\pm$ 0,02	0,13 $\pm$ 0,03	1,70 $\pm$ 5,07	0,15 $\pm$ 0,09	0,56 $\pm$ 0,29	0,25 $\pm$ 0,16	0,40 $\pm$ 0,31
22:1n-11	0,25 $\pm$ 0,07	0,56 $\pm$ 0,20	0,30 $\pm$ 0,12	0,54 $\pm$ 0,49	2,60 $\pm$ 1,58	0,86 $\pm$ 1,22	1,13 $\pm$ 0,99
24:1n-9	0,24 $\pm$ 0,04	0,23 $\pm$ 0,02	0,20 $\pm$ 0,01	0,16 $\pm$ 0,04	0,30 $\pm$ 0,09	0,23 $\pm$ 0,09	0,23 $\pm$ 0,08
16:2n-4	0,07 $\pm$ 0,02	0,07 $\pm$ 0,01	0,08 $\pm$ 0,02	0,31 $\pm$ 0,11	0,25 $\pm$ 0,16	0,11 $\pm$ 0,07	0,12 $\pm$ 0,06
16:3n-3	0,13 $\pm$ 0,04	0,17 $\pm$ 0,02	0,18 $\pm$ 0,02	0,05 $\pm$ 0,02	0,19 $\pm$ 0,08	0,20 $\pm$ 0,06	0,22 $\pm$ 0,07
16:3n-4	0,02 $\pm$ 0,01	0,02 $\pm$ 0,01	0,04 $\pm$ 0,01	0,21 $\pm$ 0,10	0,11 $\pm$ 0,06	0,07 $\pm$ 0,05	0,08 $\pm$ 0,05
18:2n-4	0,08 $\pm$ 0,01	0,08 $\pm$ 0,01	0,09 $\pm$ 0,01	0,14 $\pm$ 0,05	0,17 $\pm$ 0,20	0,16 $\pm$ 0,08	0,26 $\pm$ 0,12
18:2n-6, LA	0,68 $\pm$ 0,12	0,66 $\pm$ 0,10	0,56 $\pm$ 0,08	1,43 $\pm$ 0,24	0,80 $\pm$ 0,30	0,74 $\pm$ 0,22	0,69 $\pm$ 0,32
18:3n-3, ALA	0,18 $\pm$ 0,04	0,20 $\pm$ 0,02	0,15 $\pm$ 0,02	0,41 $\pm$ 0,07	0,31 $\pm$ 0,18	0,20 $\pm$ 0,11	0,22 $\pm$ 0,12
18:3n-6	0,08 $\pm$ 0,02	0,08 $\pm$ 0,01	0,12 $\pm$ 0,03	0,17 $\pm$ 0,03	0,11 $\pm$ 0,04	0,11 $\pm$ 0,01	0,15 $\pm$ 0,03
18:4n-3 <sup>b</sup>	0,38 $\pm$ 0,18	0,42 $\pm$ 0,07	0,45 $\pm$ 0,06	1,49 $\pm$ 0,61	1,03 $\pm$ 0,54	0,78 $\pm$ 0,56	0,77 $\pm$ 0,52
20:2n-6	0,18 $\pm$ 0,03	0,16 $\pm$ 0,03	0,24 $\pm$ 0,10	0,30 $\pm$ 0,03	0,22 $\pm$ 0,05	0,24 $\pm$ 0,07	0,31 $\pm$ 0,07
20:3n-3	0,05 $\pm$ 0,01	0,05 $\pm$ 0,01	0,07 $\pm$ 0,03	0,08 $\pm$ 0,01	0,10 $\pm$ 0,02	0,08 $\pm$ 0,02	0,10 $\pm$ 0,03
20:3n-6	0,08 $\pm$ 0,02	0,07 $\pm$ 0,02	0,09 $\pm$ 0,03	$\pm$	0,06 $\pm$ 0,01	0,07 $\pm$ 0,02	0,08 $\pm$ 0,02
20:4n-3 <sup>c</sup>	0,31 $\pm$ 0,05	0,38 $\pm$ 0,05	0,38 $\pm$ 0,05	0,46 $\pm$ 0,06	0,43 $\pm$ 0,11	0,49 $\pm$ 0,20	0,37 $\pm$ 0,10
20:4n-6, AA	2,56 $\pm$ 0,57	2,00 $\pm$ 0,36	2,02 $\pm$ 0,84	1,47 $\pm$ 0,29	1,98 $\pm$ 0,81	3,01 $\pm$ 1,14	4,67 $\pm$ 2,32
20:5n-3, EPA	16,55 $\pm$ 1,09	16,69 $\pm$ 0,90	16,68 $\pm$ 5,87	17,83 $\pm$ 1,47	14,67 $\pm$ 2,37	16,65 $\pm$ 3,23	15,17 $\pm$ 3,30
21:5n-3	0,32 $\pm$ 0,04	0,34 $\pm$ 0,03	0,39 $\pm$ 0,10	0,36 $\pm$ 0,05	0,37 $\pm$ 0,06	0,34 $\pm$ 0,09	0,36 $\pm$ 0,07
22:3n-3 <sup>d</sup>	0,08 $\pm$ 0,04	0,09 $\pm$ 0,02	0,15 $\pm$ 0,08	0,08 $\pm$ 0,04	0,12 $\pm$ 0,05	0,11 $\pm$ 0,04	0,13 $\pm$ 0,03
22:5n-3	1,63 $\pm$ 0,17	1,41 $\pm$ 0,13	1,63 $\pm$ 0,25	1,29 $\pm$ 0,26	2,24 $\pm$ 0,37	1,93 $\pm$ 0,66	1,40 $\pm$ 0,24
22:5n-6	0,30 $\pm$ 0,05	0,32 $\pm$ 0,03	0,40 $\pm$ 0,16	0,26 $\pm$ 0,04	0,21 $\pm$ 0,06	0,26 $\pm$ 0,12	0,28 $\pm$ 0,11
22:6n-3, DHA	34,05 $\pm$ 1,96	34,45 $\pm$ 1,86	29,58 $\pm$ 1,87	20,50 $\pm$ 3,39	19,66 $\pm$ 5,01	20,11 $\pm$ 4,83	14,54 $\pm$ 4,21
Identified%	98,71 $\pm$ 0,19	98,78 $\pm$ 0,30	97,79 $\pm$ 1,05	97,86 $\pm$ 0,34	97,26 $\pm$ 0,91	97,09 $\pm$ 0,77	95,14 $\pm$ 1,22
SAFA	23,82 $\pm$ 0,68	23,52 $\pm$ 0,62	24,71 $\pm$ 0,79	22,29 $\pm$ 1,27	24,54 $\pm$ 1,44	20,87 $\pm$ 0,65	20,88 $\pm$ 1,06
MUFA	16,98 $\pm$ 1,25	17,42 $\pm$ 1,06	18,97 $\pm$ 4,44	28,30 $\pm$ 4,00	28,83 $\pm$ 8,40	29,86 $\pm$ 5,44	31,68 $\pm$ 7,96
PUFA	57,91 $\pm$ 1,20	57,85 $\pm$ 0,88	54,11 $\pm$ 4,59	47,26 $\pm$ 3,47	43,89 $\pm$ 6,89	46,36 $\pm$ 4,94	42,58 $\pm$ 7,22
n-6	3,89 $\pm$ 0,58	3,30 $\pm$ 0,49	3,36 $\pm$ 1,07	3,62 $\pm$ 0,30	3,44 $\pm$ 0,77	4,42 $\pm$ 1,12	6,19 $\pm$ 2,23
n-3	53,78 $\pm$ 1,28	54,31 $\pm$ 1,28	49,78 $\pm$ 5,74	42,95 $\pm$ 3,54	39,81 $\pm$ 6,34	41,28 $\pm$ 4,23	34,69 $\pm$ 6,17
n-6/n-3	0,07 $\pm$ 0,01	0,06 $\pm$ 0,01	0,07 $\pm$ 0,02	0,08 $\pm$ 0,01	0,09 $\pm$ 0,01	0,11 $\pm$ 0,02	0,18 $\pm$ 0,06

Table 3 - continued

Fish	Deepwater redbfish	Golden redbfish	Capelin	Greenland halibut	Arctic char	Atlantic salmon
Fatty acid						
14:0	2,62 ± 0,61	3,03 ± 0,48	3,75 ± 0,61	3,91 ± 0,76	3,54 ± 0,57	4,44 ± 0,27
Pristanic acid	0,10 ± 0,02	0,10 ± 0,04	0,13 ± 0,03	0,16 ± 0,03	0,03 ± 0,01	0,02 ± 0,01
16:0	11,74 ± 1,60	12,90 ± 1,79	20,28 ± 1,63	13,97 ± 3,00	14,80 ± 0,70	14,44 ± 1,08
Phytanic acid	0,45 ± 0,10	0,57 ± 0,12	0,99 ± 0,16	0,70 ± 0,28	0,47 ± 0,07	0,35 ± 0,04
17:0	0,17 ± 0,04	0,17 ± 0,02	0,09 ± 0,04	0,10 ± 0,03	0,15 ± 0,02	0,20 ± 0,04
ai-17:0	0,07 ± 0,01	0,09 ± 0,02	0,06 ± 0,00	0,09 ± 0,02	0,11 ± 0,01	0,09 ± 0,02
18:0	3,00 ± 0,61	2,98 ± 0,43	2,03 ± 0,33	2,29 ± 0,92	3,61 ± 0,30	2,48 ± 0,43
iso-18:0	0,13 ± 0,03	0,14 ± 0,02	0,07 ± 0,02	0,08 ± 0,02	0,08 ± 0,01	0,11 ± 0,02
14:1n-5	0,22 ± 0,06	0,26 ± 0,05	0,23 ± 0,06	0,26 ± 0,04	0,23 ± 0,02	0,30 ± 0,06
16:1n-7	5,18 ± 1,69	5,38 ± 1,23	6,04 ± 1,70	10,00 ± 1,77	5,61 ± 0,77	5,78 ± 0,80
16:1n-9	0,19 ± 0,03	0,18 ± 0,03	0,15 ± 0,01	0,21 ± 0,03	0,20 ± 0,01	0,21 ± 0,03
16:1n-11	0,28 ± 0,02	0,27 ± 0,04	0,21 ± 0,03	0,26 ± 0,07	0,24 ± 0,04	0,27 ± 0,01
18:1n-5	0,45 ± 0,07	0,44 ± 0,07	0,24 ± 0,06	0,35 ± 0,13	0,33 ± 0,06	0,47 ± 0,04
18:1n-7	3,19 ± 0,55	3,99 ± 0,62	6,34 ± 1,36	6,45 ± 4,32	3,19 ± 0,46	3,00 ± 0,17
18:1n-9	10,44 ± 2,00	11,78 ± 1,96	14,71 ± 2,49	16,92 ± 5,74	19,44 ± 1,50	10,39 ± 1,72
18:1n-11	1,14 ± 0,35	0,93 ± 0,21	0,26 ± 0,12	0,79 ± 0,60	0,31 ± 0,13	0,87 ± 0,10
18:1n-13 <sup>a</sup>	0,26 ± 0,10	0,30 ± 0,07	0,29 ± 0,05	0,21 ± 0,09	0,65 ± 0,32	0,36 ± 0,10
20:1n-7	0,65 ± 0,25	0,69 ± 0,25	0,24 ± 0,05	1,14 ± 0,31	0,31 ± 0,05	0,37 ± 0,05
20:1n-9	11,45 ± 2,69	9,13 ± 1,43	2,60 ± 1,26	11,42 ± 3,91	3,12 ± 1,22	9,82 ± 1,42
20:1n-11	1,49 ± 0,36	1,18 ± 0,22	0,32 ± 0,10	1,28 ± 0,65	0,50 ± 0,08	1,19 ± 0,08
22:1n-9	1,80 ± 0,54	1,71 ± 0,41	0,32 ± 0,12	1,79 ± 0,74	0,38 ± 0,10	1,02 ± 0,11
22:1n-11	11,22 ± 2,87	9,48 ± 2,15	2,81 ± 1,63	8,86 ± 3,90	1,82 ± 1,03	8,32 ± 1,45
24:1n-9	0,66 ± 0,16	0,63 ± 0,08	0,29 ± 0,16	0,64 ± 0,24	0,40 ± 0,09	0,66 ± 0,04
16:2n-4	0,24 ± 0,09	0,28 ± 0,05	0,41 ± 0,13	0,36 ± 0,13	0,39 ± 0,15	0,30 ± 0,09
16:3n-3	0,08 ± 0,06	0,09 ± 0,04	0,04 ± 0,03	0,03 ± 0,02	0,04 ± 0,01	0,03 ± 0,00
16:3n-4	0,11 ± 0,04	0,13 ± 0,03	0,13 ± 0,04	0,13 ± 0,05	0,17 ± 0,08	0,12 ± 0,05
18:2n-4	0,10 ± 0,02	0,10 ± 0,02	0,09 ± 0,02	0,16 ± 0,17	0,14 ± 0,02	0,11 ± 0,02
18:2n-6, LA	1,30 ± 0,23	1,22 ± 0,10	1,12 ± 0,13	0,68 ± 0,26	1,47 ± 0,16	1,33 ± 0,16
18:3n-3, ALA	0,39 ± 0,08	0,39 ± 0,08	0,45 ± 0,13	0,27 ± 0,13	0,89 ± 0,07	0,73 ± 0,12
18:3n-6	0,12 ± 0,01	0,13 ± 0,01	0,14 ± 0,04	0,12 ± 0,03	0,14 ± 0,02	0,10 ± 0,01
18:4n-3 <sup>b</sup>	0,91 ± 0,31	1,08 ± 0,19	1,58 ± 0,37	0,89 ± 0,49	2,63 ± 0,45	1,73 ± 0,60
20:2n-6	0,22 ± 0,05	0,20 ± 0,02	0,15 ± 0,01	0,26 ± 0,03	0,35 ± 0,03	0,33 ± 0,05
20:3n-3	0,09 ± 0,04	0,10 ± 0,03	0,04 ± 0,01	0,10 ± 0,03	0,15 ± 0,01	0,19 ± 0,06
20:3n-6	0,11 ± 0,04	0,08 ± 0,02	0,03 ± 0,00	0,06 ± 0,02	0,10 ± 0,01	0,10 ± 0,02
20:4n-3 <sup>c</sup>	0,46 ± 0,07	0,44 ± 0,09	0,30 ± 0,07	0,33 ± 0,12	1,57 ± 0,19	1,35 ± 0,22
20:4n-6, AA	0,86 ± 0,41	0,75 ± 0,15	0,40 ± 0,09	0,38 ± 0,12	0,58 ± 0,09	0,36 ± 0,08
20:5n-3, EPA	7,64 ± 1,76	8,44 ± 1,48	13,95 ± 1,71	4,90 ± 1,27	10,09 ± 1,34	7,94 ± 0,26
21:5n-3	0,25 ± 0,05	0,31 ± 0,03	0,45 ± 0,03	0,24 ± 0,13	0,47 ± 0,06	0,42 ± 0,04
22:3n-3 <sup>d</sup>	0,03 ± 0,01	0,04 ± 0,01	±	0,04 ± 0,01	0,08 ± 0,01	0,05 ± 0,01
22:5n-3	0,76 ± 0,11	0,77 ± 0,10	0,58 ± 0,13	0,79 ± 0,15	2,50 ± 0,08	2,49 ± 0,34
22:5n-6	0,23 ± 0,13	0,21 ± 0,05	0,08 ± 0,03	0,05 ± 0,01	0,09 ± 0,01	0,09 ± 0,01
22:6n-3, DHA	15,83 ± 5,97	15,25 ± 3,57	14,99 ± 4,23	4,65 ± 0,82	13,84 ± 1,71	12,96 ± 1,76
Identified%	98,11 ± 0,33	97,92 ± 0,22	98,58 ± 0,21	98,14 ± 1,03	98,16 ± 0,10	97,90 ± 0,15
SAFA	19,02 ± 1,99	20,80 ± 2,07	28,04 ± 1,64	22,51 ± 3,99	23,67 ± 1,19	23,14 ± 1,32
MUFA	49,07 ± 9,28	46,86 ± 6,23	35,50 ± 4,96	61,03 ± 6,31	37,17 ± 1,82	43,55 ± 2,18
PUFA	30,02 ± 7,71	30,26 ± 4,56	35,04 ± 5,34	14,60 ± 2,77	37,33 ± 1,22	31,21 ± 1,56
n-6	2,86 ± 0,76	2,59 ± 0,22	1,94 ± 0,18	1,56 ± 0,28	2,74 ± 0,23	2,31 ± 0,14
n-3	26,68 ± 7,09	27,12 ± 4,44	32,45 ± 5,28	12,37 ± 2,68	33,77 ± 1,18	28,26 ± 1,62
n-6/n-3	0,11 ± 0,01	0,10 ± 0,01	0,06 ± 0,01	0,13 ± 0,04	0,08 ± 0,01	0,08 ± 0,00

**Table 4.**

Fatty acid composition of total lipid of seabirds (muscle). Individual fatty acids are given as mass% (mean  $\pm$  stdev) of total fatty acids.

<b>Seabirds</b>					
Fatty acid	Common eider	B. guillemot	King eider	Little auk	Kittiwake
14:0	1,01 $\pm$ 0,21	1,52 $\pm$ 0,54	1,62 $\pm$ 0,70	1,99 $\pm$ 0,32	2,50 $\pm$ 0,29
Pristanic acid	0,02 $\pm$ 0,00	0,05 $\pm$ 0,03	0,32 $\pm$ 0,82	0,10 $\pm$ 0,04	0,04 $\pm$ 0,02
16:0	16,69 $\pm$ 1,19	14,09 $\pm$ 1,78	15,27 $\pm$ 1,36	16,90 $\pm$ 1,16	14,62 $\pm$ 1,92
Phytanic acid	0,31 $\pm$ 0,05	0,48 $\pm$ 0,12	0,43 $\pm$ 0,07	1,06 $\pm$ 0,29	0,38 $\pm$ 0,10
17:0	0,48 $\pm$ 0,06	0,14 $\pm$ 0,01	0,42 $\pm$ 0,07	0,17 $\pm$ 0,02	0,22 $\pm$ 0,04
ai-17:0	0,30 $\pm$ 0,05	0,06 $\pm$ 0,01	0,25 $\pm$ 0,07	0,07 $\pm$ 0,01	0,09 $\pm$ 0,02
18:0	12,47 $\pm$ 0,68	11,21 $\pm$ 1,15	13,90 $\pm$ 1,37	11,41 $\pm$ 0,83	9,60 $\pm$ 1,25
iso-18:0	0,23 $\pm$ 0,05	0,10 $\pm$ 0,01	0,19 $\pm$ 0,04	0,10 $\pm$ 0,02	0,11 $\pm$ 0,02
14:1n-5	0,14 $\pm$ 0,03	0,13 $\pm$ 0,06	0,14 $\pm$ 0,04	0,13 $\pm$ 0,02	0,19 $\pm$ 0,03
16:1n-7	3,80 $\pm$ 1,04	3,30 $\pm$ 0,91	3,92 $\pm$ 1,70	3,86 $\pm$ 0,42	4,93 $\pm$ 0,87
16:1n-9	0,21 $\pm$ 0,05	0,10 $\pm$ 0,02	0,16 $\pm$ 0,03	0,10 $\pm$ 0,02	0,17 $\pm$ 0,01
16:1n-11	0,18 $\pm$ 0,05	0,11 $\pm$ 0,03	0,15 $\pm$ 0,04	0,13 $\pm$ 0,02	0,24 $\pm$ 0,03
18:1n-5	0,22 $\pm$ 0,04	0,13 $\pm$ 0,04	0,19 $\pm$ 0,04	0,24 $\pm$ 0,05	0,31 $\pm$ 0,04
18:1n-7	4,04 $\pm$ 0,48	4,57 $\pm$ 0,59	4,53 $\pm$ 0,58	4,71 $\pm$ 0,30	3,18 $\pm$ 0,76
18:1n-9	18,39 $\pm$ 1,65	17,08 $\pm$ 0,56	13,37 $\pm$ 2,76	17,48 $\pm$ 2,21	15,74 $\pm$ 1,95
18:1n-11	0,09 $\pm$ 0,04	0,47 $\pm$ 0,20	0,06 $\pm$ 0,01	0,28 $\pm$ 0,08	1,16 $\pm$ 0,32
18:1n-13 <sup>a</sup>	0,09 $\pm$ 0,02	0,13 $\pm$ 0,03	0,24 $\pm$ 0,15	0,11 $\pm$ 0,01	0,19 $\pm$ 0,02
20:1n-7	0,75 $\pm$ 0,18	0,23 $\pm$ 0,05	0,81 $\pm$ 0,23	0,25 $\pm$ 0,07	0,35 $\pm$ 0,10
20:1n-9	1,08 $\pm$ 0,34	3,96 $\pm$ 1,80	0,76 $\pm$ 0,16	4,05 $\pm$ 0,96	9,07 $\pm$ 2,66
20:1n-11	0,47 $\pm$ 0,10	1,12 $\pm$ 0,57	0,89 $\pm$ 0,38	0,71 $\pm$ 0,12	2,56 $\pm$ 0,92
22:1n-9	0,16 $\pm$ 0,08	0,30 $\pm$ 0,11	0,16 $\pm$ 0,05	0,43 $\pm$ 0,17	0,60 $\pm$ 0,21
22:1n-11	0,20 $\pm$ 0,19	2,80 $\pm$ 1,43	0,45 $\pm$ 0,23	3,24 $\pm$ 1,37	6,97 $\pm$ 2,40
24:1n-9	0,05 $\pm$ 0,02	0,09 $\pm$ 0,02	0,07 $\pm$ 0,02	0,09 $\pm$ 0,03	0,14 $\pm$ 0,03
16:2n-4	0,10 $\pm$ 0,03	0,24 $\pm$ 0,05	0,17 $\pm$ 0,08	0,25 $\pm$ 0,04	0,23 $\pm$ 0,04
16:3n-3	0,19 $\pm$ 0,07	0,24 $\pm$ 0,06	0,19 $\pm$ 0,04	0,14 $\pm$ 0,07	0,12 $\pm$ 0,04
16:3n-4	0,04 $\pm$ 0,02	0,05 $\pm$ 0,01	0,08 $\pm$ 0,05	0,04 $\pm$ 0,01	0,06 $\pm$ 0,01
18:2n-4	0,12 $\pm$ 0,02	0,06 $\pm$ 0,01	0,23 $\pm$ 0,07	0,07 $\pm$ 0,01	0,07 $\pm$ 0,01
18:2n-6, LA	3,03 $\pm$ 0,54	3,22 $\pm$ 0,58	2,08 $\pm$ 0,41	3,67 $\pm$ 0,51	2,55 $\pm$ 0,31
18:3n-3, ALA	0,82 $\pm$ 0,21	0,89 $\pm$ 0,12	0,67 $\pm$ 0,18	0,97 $\pm$ 0,18	0,76 $\pm$ 0,12
18:3n-6	0,10 $\pm$ 0,02	0,07 $\pm$ 0,01	0,11 $\pm$ 0,03	0,07 $\pm$ 0,01	0,11 $\pm$ 0,02
18:4n-3 <sup>b</sup>	0,38 $\pm$ 0,20	0,30 $\pm$ 0,09	1,02 $\pm$ 0,46	0,34 $\pm$ 0,08	0,74 $\pm$ 0,17
20:2n-6	0,54 $\pm$ 0,13	0,18 $\pm$ 0,02	0,59 $\pm$ 0,18	0,18 $\pm$ 0,03	0,22 $\pm$ 0,03
20:3n-3	0,13 $\pm$ 0,07	0,04 $\pm$ 0,01	0,16 $\pm$ 0,10	0,04 $\pm$ 0,01	0,07 $\pm$ 0,01
20:3n-6	0,25 $\pm$ 0,04	0,10 $\pm$ 0,01	0,21 $\pm$ 0,07	0,09 $\pm$ 0,01	0,13 $\pm$ 0,02
20:4n-3 <sup>c</sup>	0,25 $\pm$ 0,09	0,19 $\pm$ 0,03	0,44 $\pm$ 0,13	0,19 $\pm$ 0,02	0,28 $\pm$ 0,07
20:4n-6, AA	10,48 $\pm$ 1,83	3,54 $\pm$ 0,86	9,11 $\pm$ 4,56	4,61 $\pm$ 0,97	2,95 $\pm$ 0,35
20:5n-3, EPA	5,35 $\pm$ 0,77	12,14 $\pm$ 1,86	8,42 $\pm$ 1,92	9,30 $\pm$ 1,11	7,09 $\pm$ 1,08
21:5n-3	0,11 $\pm$ 0,02	0,08 $\pm$ 0,02	0,18 $\pm$ 0,09	0,07 $\pm$ 0,01	0,11 $\pm$ 0,02
22:3n-3 <sup>d</sup>	0,73 $\pm$ 0,17	0,07 $\pm$ 0,02	0,61 $\pm$ 0,26	0,04 $\pm$ 0,01	0,05 $\pm$ 0,01
22:5n-3	0,71 $\pm$ 0,09	0,54 $\pm$ 0,05	0,98 $\pm$ 0,16	0,52 $\pm$ 0,10	0,68 $\pm$ 0,13
22:5n-6	0,26 $\pm$ 0,04	0,12 $\pm$ 0,02	0,31 $\pm$ 0,05	0,06 $\pm$ 0,01	0,12 $\pm$ 0,03
22:6n-3, DHA	5,15 $\pm$ 0,71	6,27 $\pm$ 0,88	5,90 $\pm$ 1,31	6,68 $\pm$ 0,88	5,22 $\pm$ 1,37
Identified%	93,08 $\pm$ 0,46	92,80 $\pm$ 1,25	93,37 $\pm$ 1,24	96,41 $\pm$ 0,70	96,77 $\pm$ 0,38
SAFA	32,44 $\pm$ 1,49	28,24 $\pm$ 2,40	33,26 $\pm$ 1,34	32,36 $\pm$ 0,85	28,23 $\pm$ 3,08
MUFA	31,54 $\pm$ 1,78	36,17 $\pm$ 4,26	27,73 $\pm$ 3,92	36,63 $\pm$ 3,22	46,87 $\pm$ 5,48
PUFA	29,10 $\pm$ 2,05	28,39 $\pm$ 3,07	32,37 $\pm$ 3,40	27,43 $\pm$ 2,48	21,67 $\pm$ 2,72
n-6	14,68 $\pm$ 2,10	7,23 $\pm$ 1,33	12,44 $\pm$ 5,09	8,67 $\pm$ 1,46	6,07 $\pm$ 0,56
n-3	13,94 $\pm$ 1,14	20,81 $\pm$ 2,05	19,00 $\pm$ 2,71	18,34 $\pm$ 1,76	15,24 $\pm$ 2,52
n-6/n-3	1,06 $\pm$ 0,19	0,35 $\pm$ 0,05	0,69 $\pm$ 0,36	0,48 $\pm$ 0,09	0,41 $\pm$ 0,07

**Table 5.**Fatty acid composition of total lipid of marine mammal muscle. Individual fatty acids are given as mass% (mean  $\pm$  stdev) of total fatty acids.

<b>Marine mammals</b>							
Fatty acid	Walrus	Narwhal	Beluga	Hooded seal	Harp seal	Ringed seal	Minke whale
14:0	1,44 $\pm$ 0,73	3,23 $\pm$ 0,62	2,87 $\pm$ 0,48	2,97 $\pm$ 0,60	3,53 $\pm$ 1,11	3,71 $\pm$ 0,52	5,43 $\pm$ 1,27
Pristanic acid	0,13 $\pm$ 0,04	0,33 $\pm$ 0,07	0,23 $\pm$ 0,07	0,12 $\pm$ 0,06	0,06 $\pm$ 0,03	0,08 $\pm$ 0,03	0,11 $\pm$ 0,01
16:0	13,74 $\pm$ 0,83	12,58 $\pm$ 0,62	11,33 $\pm$ 0,83	14,59 $\pm$ 0,75	12,54 $\pm$ 1,06	9,84 $\pm$ 0,52	13,00 $\pm$ 0,67
Phytanic acid	0,15 $\pm$ 0,03	0,32 $\pm$ 0,06	0,35 $\pm$ 0,04	0,71 $\pm$ 0,24	0,29 $\pm$ 0,04	0,23 $\pm$ 0,03	0,42 $\pm$ 0,06
17:0	0,32 $\pm$ 0,04	0,21 $\pm$ 0,06	0,32 $\pm$ 0,13	0,19 $\pm$ 0,03	0,20 $\pm$ 0,02	0,15 $\pm$ 0,02	0,23 $\pm$ 0,04
ai-17:0	0,15 $\pm$ 0,04	0,05 $\pm$ 0,01	0,08 $\pm$ 0,01	0,10 $\pm$ 0,01	0,10 $\pm$ 0,01	0,07 $\pm$ 0,01	0,08 $\pm$ 0,00
18:0	8,59 $\pm$ 2,22	6,61 $\pm$ 1,11	6,20 $\pm$ 1,13	6,10 $\pm$ 1,08	6,08 $\pm$ 1,66	3,47 $\pm$ 0,77	3,40 $\pm$ 1,30
iso-18:0	0,22 $\pm$ 0,05	0,23 $\pm$ 0,03	0,19 $\pm$ 0,03	0,21 $\pm$ 0,04	0,16 $\pm$ 0,03	0,20 $\pm$ 0,04	0,12 $\pm$ 0,04
14:1n-5	0,17 $\pm$ 0,13	0,46 $\pm$ 0,15	0,30 $\pm$ 0,06	0,27 $\pm$ 0,03	0,26 $\pm$ 0,12	0,21 $\pm$ 0,08	0,39 $\pm$ 0,07
16:1n-7	8,40 $\pm$ 3,84	9,75 $\pm$ 1,66	7,48 $\pm$ 0,84	6,07 $\pm$ 0,82	4,49 $\pm$ 1,00	8,18 $\pm$ 0,92	5,25 $\pm$ 0,77
16:1n-9	0,24 $\pm$ 0,07	0,33 $\pm$ 0,11	0,32 $\pm$ 0,07	0,24 $\pm$ 0,04	0,23 $\pm$ 0,05	0,25 $\pm$ 0,04	0,32 $\pm$ 0,08
16:1n-11	0,22 $\pm$ 0,04	0,32 $\pm$ 0,10	0,26 $\pm$ 0,05	0,22 $\pm$ 0,04	0,23 $\pm$ 0,05	0,34 $\pm$ 0,04	0,29 $\pm$ 0,03
18:1n-5	0,25 $\pm$ 0,12	0,29 $\pm$ 0,05	0,38 $\pm$ 0,04	0,37 $\pm$ 0,05	0,37 $\pm$ 0,04	0,53 $\pm$ 0,06	0,45 $\pm$ 0,04
18:1n-7	8,09 $\pm$ 0,37	3,60 $\pm$ 0,19	4,26 $\pm$ 0,24	4,98 $\pm$ 0,29	3,02 $\pm$ 0,27	3,04 $\pm$ 0,19	2,60 $\pm$ 0,27
18:1n-9	12,91 $\pm$ 2,19	15,45 $\pm$ 1,17	14,20 $\pm$ 1,44	21,09 $\pm$ 0,62	12,69 $\pm$ 1,12	10,62 $\pm$ 0,94	11,57 $\pm$ 2,64
18:1n-11	0,09 $\pm$ 0,06	1,69 $\pm$ 0,37	1,99 $\pm$ 0,35	1,61 $\pm$ 0,41	1,90 $\pm$ 0,51	3,22 $\pm$ 0,25	1,21 $\pm$ 0,24
18:1n-13 <sup>a</sup>	0,29 $\pm$ 0,16	0,11 $\pm$ 0,02	0,12 $\pm$ 0,02	0,19 $\pm$ 0,03	0,37 $\pm$ 0,15	0,23 $\pm$ 0,05	0,59 $\pm$ 0,15
20:1n-7	1,83 $\pm$ 0,56	0,57 $\pm$ 0,14	0,71 $\pm$ 0,12	0,56 $\pm$ 0,09	0,29 $\pm$ 0,04	0,93 $\pm$ 0,07	0,29 $\pm$ 0,04
20:1n-9	0,79 $\pm$ 0,22	7,11 $\pm$ 1,57	8,98 $\pm$ 1,46	6,83 $\pm$ 1,46	10,71 $\pm$ 2,25	14,10 $\pm$ 1,60	12,14 $\pm$ 3,04
20:1n-11	0,67 $\pm$ 0,21	1,96 $\pm$ 0,29	2,00 $\pm$ 0,31	1,64 $\pm$ 0,24	1,74 $\pm$ 0,24	3,09 $\pm$ 0,32	1,16 $\pm$ 0,23
22:1n-9	0,06 $\pm$ 0,02	1,03 $\pm$ 0,29	1,25 $\pm$ 0,26	0,87 $\pm$ 0,26	0,56 $\pm$ 0,13	1,29 $\pm$ 0,19	0,87 $\pm$ 0,21
22:1n-11	0,09 $\pm$ 0,04	6,26 $\pm$ 1,57	7,25 $\pm$ 1,68	3,99 $\pm$ 1,28	5,62 $\pm$ 1,67	8,73 $\pm$ 1,77	10,53 $\pm$ 3,12
24:1n-9	0,03 $\pm$ 0,01	0,32 $\pm$ 0,06	0,42 $\pm$ 0,09	0,51 $\pm$ 0,16	0,24 $\pm$ 0,04	0,22 $\pm$ 0,06	0,44 $\pm$ 0,07
16:2n-4	0,17 $\pm$ 0,09	0,28 $\pm$ 0,05	0,27 $\pm$ 0,04	0,25 $\pm$ 0,04	0,31 $\pm$ 0,09	0,45 $\pm$ 0,05	0,39 $\pm$ 0,08
16:3n-3	0,23 $\pm$ 0,11	0,21 $\pm$ 0,09	0,27 $\pm$ 0,12	0,07 $\pm$ 0,04	0,34 $\pm$ 0,20	0,10 $\pm$ 0,04	0,03 $\pm$ 0,01
16:3n-4	0,32 $\pm$ 0,12	0,08 $\pm$ 0,01	0,08 $\pm$ 0,01	0,09 $\pm$ 0,01	0,15 $\pm$ 0,06	0,14 $\pm$ 0,03	0,18 $\pm$ 0,02
18:2n-4	0,25 $\pm$ 0,03	0,09 $\pm$ 0,01	0,10 $\pm$ 0,01	0,11 $\pm$ 0,01	0,11 $\pm$ 0,01	0,11 $\pm$ 0,01	0,10 $\pm$ 0,02
18:2n-6, LA	0,89 $\pm$ 0,14	1,09 $\pm$ 0,07	1,16 $\pm$ 0,08	1,68 $\pm$ 0,23	2,57 $\pm$ 0,58	1,85 $\pm$ 0,19	1,72 $\pm$ 0,10
18:3n-3, ALA	0,08 $\pm$ 0,04	0,19 $\pm$ 0,03	0,25 $\pm$ 0,03	0,29 $\pm$ 0,04	0,64 $\pm$ 0,13	0,48 $\pm$ 0,08	0,67 $\pm$ 0,10
18:3n-6	0,11 $\pm$ 0,05	0,12 $\pm$ 0,02	0,12 $\pm$ 0,01	0,09 $\pm$ 0,00	0,10 $\pm$ 0,01	0,12 $\pm$ 0,02	0,10 $\pm$ 0,01
18:4n-3 <sup>b</sup>	0,24 $\pm$ 0,17	0,30 $\pm$ 0,06	0,45 $\pm$ 0,08	0,56 $\pm$ 0,13	1,46 $\pm$ 0,58	0,85 $\pm$ 0,11	1,88 $\pm$ 0,32
20:2n-6	0,34 $\pm$ 0,03	0,20 $\pm$ 0,03	0,23 $\pm$ 0,02	0,21 $\pm$ 0,02	0,18 $\pm$ 0,01	0,23 $\pm$ 0,01	0,24 $\pm$ 0,04
20:3n-3	0,03 $\pm$ 0,01	0,04 $\pm$ 0,01	0,06 $\pm$ 0,01	0,07 $\pm$ 0,01	0,06 $\pm$ 0,01	0,06 $\pm$ 0,01	0,06 $\pm$ 0,01
20:3n-6	$\pm$	0,15 $\pm$ 0,03	0,13 $\pm$ 0,02	0,09 $\pm$ 0,02	$\pm$	$\pm$	0,08 $\pm$ 0,01
20:4n-3 <sup>c</sup>	0,27 $\pm$ 0,07	0,19 $\pm$ 0,03	0,30 $\pm$ 0,05	0,31 $\pm$ 0,05	0,42 $\pm$ 0,08	0,36 $\pm$ 0,03	0,75 $\pm$ 0,07
20:4n-6, AA	6,12 $\pm$ 1,98	1,99 $\pm$ 0,55	1,64 $\pm$ 0,46	2,81 $\pm$ 1,10	2,61 $\pm$ 1,17	1,46 $\pm$ 0,36	0,59 $\pm$ 0,32
20:5n-3, EPA	11,20 $\pm$ 2,43	6,45 $\pm$ 1,09	5,95 $\pm$ 1,03	6,09 $\pm$ 1,00	7,36 $\pm$ 1,27	5,75 $\pm$ 0,72	7,44 $\pm$ 1,61
21:5n-3	0,22 $\pm$ 0,11	0,06 $\pm$ 0,01	0,10 $\pm$ 0,02	0,15 $\pm$ 0,03	0,25 $\pm$ 0,08	0,22 $\pm$ 0,02	0,30 $\pm$ 0,06
22:3n-3 <sup>d</sup>	0,21 $\pm$ 0,02	0,05 $\pm$ 0,02	0,07 $\pm$ 0,04	0,07 $\pm$ 0,02	0,05 $\pm$ 0,01	0,05 $\pm$ 0,01	0,14 $\pm$ 0,01
22:5n-3	3,88 $\pm$ 0,40	0,91 $\pm$ 0,13	1,46 $\pm$ 0,20	1,23 $\pm$ 0,21	2,30 $\pm$ 0,64	2,67 $\pm$ 0,34	1,60 $\pm$ 0,07
22:5n-6	0,09 $\pm$ 0,01	0,05 $\pm$ 0,01	0,08 $\pm$ 0,01	0,06 $\pm$ 0,02	0,10 $\pm$ 0,02	0,08 $\pm$ 0,01	0,06 $\pm$ 0,01
22:6n-3, DHA	5,14 $\pm$ 0,52	5,80 $\pm$ 0,60	7,77 $\pm$ 1,21	4,46 $\pm$ 1,16	8,61 $\pm$ 1,22	7,25 $\pm$ 0,54	5,97 $\pm$ 1,56
Identified%	92,75 $\pm$ 1,50	94,97 $\pm$ 0,88	95,46 $\pm$ 1,07	95,20 $\pm$ 1,46	96,61 $\pm$ 0,77	97,11 $\pm$ 0,31	95,52 $\pm$ 1,17
SAFA	27,20 $\pm$ 2,64	26,14 $\pm$ 1,42	23,85 $\pm$ 1,46	26,01 $\pm$ 0,60	24,88 $\pm$ 1,85	18,80 $\pm$ 1,03	24,05 $\pm$ 0,98
MUFA	35,39 $\pm$ 4,42	50,38 $\pm$ 4,10	50,87 $\pm$ 4,09	50,25 $\pm$ 3,52	43,76 $\pm$ 3,91	55,75 $\pm$ 2,61	48,80 $\pm$ 5,13
PUFA	30,17 $\pm$ 1,50	18,44 $\pm$ 2,02	20,73 $\pm$ 2,55	18,93 $\pm$ 2,33	27,97 $\pm$ 2,30	22,56 $\pm$ 1,38	22,66 $\pm$ 3,07
n-6	7,62 $\pm$ 2,00	3,67 $\pm$ 0,58	3,42 $\pm$ 0,50	4,97 $\pm$ 1,24	5,57 $\pm$ 1,70	3,76 $\pm$ 0,51	2,81 $\pm$ 0,33
n-3	21,74 $\pm$ 2,49	14,30 $\pm$ 1,51	16,82 $\pm$ 2,25	13,48 $\pm$ 1,63	21,79 $\pm$ 2,03	18,07 $\pm$ 1,00	19,13 $\pm$ 2,94
n-6/n-3	0,36 $\pm$ 0,13	0,26 $\pm$ 0,02	0,20 $\pm$ 0,02	0,37 $\pm$ 0,09	0,26 $\pm$ 0,09	0,21 $\pm$ 0,02	0,15 $\pm$ 0,02

**Table 6.**Fatty acid composition of total lipid of marine mammals blubber. Individual fatty acids are given as mass% (mean  $\pm$  stdev) of total fatty acids.

<b>Marine mammals</b>							
Fatty acid	Walrus	Narwhal	Beluga	Hooded seal	Harp seal	Ringed seal	Minke whale
14:0	2,86 $\pm$ 0,41	4,81 $\pm$ 0,23	4,61 $\pm$ 0,29	4,18 $\pm$ 0,42	5,13 $\pm$ 0,29	3,92 $\pm$ 0,30	5,57 $\pm$ 0,68
Pristanic acid	0,03 $\pm$ 0,01	0,13 $\pm$ 0,07	0,12 $\pm$ 0,06	0,04 $\pm$ 0,01	$\pm$	0,01 $\pm$	0,09 $\pm$ 0,02
16:0	9,17 $\pm$ 1,62	6,97 $\pm$ 0,64	6,79 $\pm$ 0,75	10,31 $\pm$ 1,10	7,85 $\pm$ 0,77	6,09 $\pm$ 0,54	9,65 $\pm$ 1,11
Phytanic acid	0,19 $\pm$ 0,03	0,29 $\pm$ 0,02	0,39 $\pm$ 0,07	0,52 $\pm$ 0,08	0,36 $\pm$ 0,08	0,18 $\pm$ 0,03	0,51 $\pm$ 0,13
17:0	0,21 $\pm$ 0,04	0,13 $\pm$ 0,01	0,22 $\pm$ 0,12	0,20 $\pm$ 0,03	0,15 $\pm$ 0,03	0,08 $\pm$ 0,02	0,24 $\pm$ 0,08
ai-17:0	0,32 $\pm$ 0,05	0,09 $\pm$ 0,01	0,09 $\pm$ 0,02	0,13 $\pm$ 0,03	0,09 $\pm$ 0,01	0,06 $\pm$ 0,01	0,08 $\pm$ 0,02
18:0	1,33 $\pm$ 0,34	1,28 $\pm$ 0,23	1,27 $\pm$ 0,20	2,16 $\pm$ 0,23	1,01 $\pm$ 0,14	0,67 $\pm$ 0,13	1,82 $\pm$ 0,46
iso-18:0	0,26 $\pm$ 0,06	0,07 $\pm$ 0,01	0,07 $\pm$ 0,01	0,12 $\pm$ 0,03	0,10 $\pm$ 0,02	0,06 $\pm$ 0,01	0,11 $\pm$ 0,02
14:1n-5	0,73 $\pm$ 0,14	2,18 $\pm$ 0,52	1,81 $\pm$ 0,54	0,56 $\pm$ 0,07	0,67 $\pm$ 0,15	0,98 $\pm$ 0,23	0,59 $\pm$ 0,02
16:1n-7	19,58 $\pm$ 2,45	20,90 $\pm$ 1,75	18,39 $\pm$ 1,86	9,91 $\pm$ 0,56	11,86 $\pm$ 1,73	18,10 $\pm$ 3,05	7,23 $\pm$ 0,31
16:1n-9	0,41 $\pm$ 0,08	1,00 $\pm$ 0,26	0,95 $\pm$ 0,14	0,36 $\pm$ 0,05	0,28 $\pm$ 0,03	0,50 $\pm$ 0,07	0,22 $\pm$ 0,02
16:1n-11	0,32 $\pm$ 0,05	1,22 $\pm$ 0,30	1,14 $\pm$ 0,20	0,35 $\pm$ 0,04	0,42 $\pm$ 0,08	0,67 $\pm$ 0,07	0,30 $\pm$ 0,00
18:1n-5	0,39 $\pm$ 0,06	0,48 $\pm$ 0,03	0,45 $\pm$ 0,03	0,44 $\pm$ 0,02	0,49 $\pm$ 0,07	0,52 $\pm$ 0,05	0,46 $\pm$ 0,05
18:1n-7	9,54 $\pm$ 0,78	3,47 $\pm$ 0,19	3,42 $\pm$ 0,38	5,37 $\pm$ 0,54	3,16 $\pm$ 0,32	4,18 $\pm$ 0,52	2,48 $\pm$ 0,22
18:1n-9	16,13 $\pm$ 2,99	14,64 $\pm$ 1,18	13,40 $\pm$ 0,87	21,80 $\pm$ 1,49	12,25 $\pm$ 1,04	13,44 $\pm$ 1,13	11,43 $\pm$ 1,59
18:1n-11	0,29 $\pm$ 0,29	4,67 $\pm$ 0,26	4,23 $\pm$ 0,16	2,25 $\pm$ 0,53	3,16 $\pm$ 0,82	4,41 $\pm$ 0,71	1,49 $\pm$ 0,13
18:1n-13 <sup>a</sup>	0,58 $\pm$ 0,08	0,19 $\pm$ 0,02	0,20 $\pm$ 0,02	0,34 $\pm$ 0,07	0,70 $\pm$ 0,11	0,50 $\pm$ 0,15	0,52 $\pm$ 0,06
20:1n-7	2,85 $\pm$ 0,40	0,75 $\pm$ 0,11	0,88 $\pm$ 0,12	0,62 $\pm$ 0,06	0,42 $\pm$ 0,10	0,59 $\pm$ 0,12	0,41 $\pm$ 0,00
20:1n-9	1,34 $\pm$ 0,57	9,62 $\pm$ 0,93	11,02 $\pm$ 1,02	9,02 $\pm$ 1,26	10,09 $\pm$ 1,88	7,87 $\pm$ 1,60	14,49 $\pm$ 3,38
20:1n-11	1,17 $\pm$ 0,16	3,40 $\pm$ 0,21	2,96 $\pm$ 0,18	1,56 $\pm$ 0,19	2,03 $\pm$ 0,47	1,99 $\pm$ 0,37	1,71 $\pm$ 0,27
22:1n-9	0,05 $\pm$ 0,02	1,00 $\pm$ 0,28	1,32 $\pm$ 0,34	0,77 $\pm$ 0,06	0,60 $\pm$ 0,13	0,51 $\pm$ 0,20	1,08 $\pm$ 0,23
22:1n-11	0,11 $\pm$ 0,08	5,50 $\pm$ 1,22	6,79 $\pm$ 1,46	4,09 $\pm$ 0,82	5,27 $\pm$ 1,25	2,40 $\pm$ 1,06	12,08 $\pm$ 3,48
24:1n-9	0,03 $\pm$ 0,01	0,12 $\pm$ 0,04	0,17 $\pm$ 0,05	0,22 $\pm$ 0,04	0,18 $\pm$ 0,04	0,06 $\pm$ 0,02	0,42 $\pm$ 0,04
16:2n-4	0,30 $\pm$ 0,04	0,52 $\pm$ 0,04	0,53 $\pm$ 0,04	0,38 $\pm$ 0,03	0,55 $\pm$ 0,06	0,60 $\pm$ 0,07	0,39 $\pm$ 0,02
16:3n-3	0,04 $\pm$ 0,01	0,03 $\pm$ 0,01	0,02 $\pm$ 0,00	0,03 $\pm$ 0,00	0,01 $\pm$ 0,00	$\pm$	0,04 $\pm$ 0,00
16:3n-4	0,08 $\pm$ 0,02	0,08 $\pm$ 0,01	0,11 $\pm$ 0,01	0,13 $\pm$ 0,01	0,26 $\pm$ 0,04	0,22 $\pm$ 0,06	0,15 $\pm$ 0,02
18:2n-4	0,29 $\pm$ 0,04	0,10 $\pm$ 0,01	0,10 $\pm$ 0,01	0,12 $\pm$ 0,02	0,11 $\pm$ 0,03	0,13 $\pm$ 0,02	0,09 $\pm$ 0,02
18:2n-6, LA	0,90 $\pm$ 0,14	0,90 $\pm$ 0,05	1,01 $\pm$ 0,05	1,33 $\pm$ 0,07	1,60 $\pm$ 0,22	1,10 $\pm$ 0,19	1,45 $\pm$ 0,07
18:3n-3, ALA	0,19 $\pm$ 0,04	0,26 $\pm$ 0,02	0,29 $\pm$ 0,04	0,49 $\pm$ 0,05	0,80 $\pm$ 0,14	0,41 $\pm$ 0,08	0,72 $\pm$ 0,04
18:3n-6	0,17 $\pm$ 0,02	0,11 $\pm$ 0,01	0,11 $\pm$ 0,01	0,13 $\pm$ 0,01	0,13 $\pm$ 0,01	0,23 $\pm$ 0,05	0,11 $\pm$ 0,01
18:4n-3 <sup>b</sup>	0,64 $\pm$ 0,09	0,35 $\pm$ 0,06	0,48 $\pm$ 0,09	1,22 $\pm$ 0,13	2,27 $\pm$ 0,49	1,12 $\pm$ 0,31	1,98 $\pm$ 0,18
20:2n-6	0,50 $\pm$ 0,06	0,19 $\pm$ 0,03	0,17 $\pm$ 0,02	0,25 $\pm$ 0,02	0,18 $\pm$ 0,02	0,17 $\pm$ 0,02	0,26 $\pm$ 0,03
20:3n-3	0,05 $\pm$ 0,02	0,04 $\pm$ 0,01	0,04 $\pm$ 0,01	0,09 $\pm$ 0,01	0,08 $\pm$ 0,02	0,05 $\pm$ 0,01	0,07 $\pm$ 0,01
20:3n-6	$\pm$	0,09 $\pm$ 0,01	0,08 $\pm$ 0,01	0,07 $\pm$ 0,02	$\pm$	$\pm$	0,08 $\pm$ 0,01
20:4n-3 <sup>c</sup>	0,67 $\pm$ 0,07	0,27 $\pm$ 0,04	0,41 $\pm$ 0,09	0,58 $\pm$ 0,07	0,64 $\pm$ 0,10	0,39 $\pm$ 0,07	0,99 $\pm$ 0,26
20:4n-6, AA	0,70 $\pm$ 0,13	0,27 $\pm$ 0,05	0,25 $\pm$ 0,05	0,49 $\pm$ 0,18	0,32 $\pm$ 0,04	0,38 $\pm$ 0,04	0,24 $\pm$ 0,04
20:5n-3, EPA	8,50 $\pm$ 2,11	3,52 $\pm$ 0,72	3,59 $\pm$ 0,65	5,58 $\pm$ 0,88	7,00 $\pm$ 0,82	9,00 $\pm$ 1,67	5,89 $\pm$ 0,95
21:5n-3	0,65 $\pm$ 0,07	0,12 $\pm$ 0,02	0,14 $\pm$ 0,02	0,39 $\pm$ 0,04	0,52 $\pm$ 0,07	0,36 $\pm$ 0,06	0,38 $\pm$ 0,02
22:3n-3 <sup>d</sup>	0,25 $\pm$ 0,04	0,03 $\pm$ 0,00	0,09 $\pm$ 0,02	0,11 $\pm$ 0,03	0,08 $\pm$ 0,04	0,07 $\pm$ 0,01	0,23 $\pm$ 0,01
22:5n-3	6,86 $\pm$ 0,73	1,61 $\pm$ 0,32	1,92 $\pm$ 0,44	2,46 $\pm$ 0,47	4,52 $\pm$ 0,63	5,75 $\pm$ 1,15	2,19 $\pm$ 0,61
22:5n-6	0,11 $\pm$ 0,02	0,04 $\pm$ 0,01	0,06 $\pm$ 0,01	0,10 $\pm$ 0,02	0,10 $\pm$ 0,02	0,06 $\pm$ 0,02	0,08 $\pm$ 0,03
22:6n-3, DHA	5,39 $\pm$ 1,00	3,31 $\pm$ 0,49	3,96 $\pm$ 0,65	7,11 $\pm$ 1,38	10,55 $\pm$ 1,48	9,00 $\pm$ 1,18	6,58 $\pm$ 2,24
Identified%	96,21 $\pm$ 0,19	98,04 $\pm$ 0,17	97,33 $\pm$ 0,72	97,98 $\pm$ 0,25	97,83 $\pm$ 0,24	98,09 $\pm$ 0,23	96,91 $\pm$ 0,30
SAFA	15,46 $\pm$ 2,17	15,71 $\pm$ 0,48	15,43 $\pm$ 0,94	18,52 $\pm$ 1,57	15,64 $\pm$ 1,10	11,67 $\pm$ 0,56	19,11 $\pm$ 1,12
MUFA	54,14 $\pm$ 3,72	70,31 $\pm$ 1,68	68,21 $\pm$ 1,46	58,12 $\pm$ 2,17	52,08 $\pm$ 3,47	57,13 $\pm$ 3,43	55,41 $\pm$ 5,60
PUFA	26,60 $\pm$ 2,31	12,02 $\pm$ 1,71	13,68 $\pm$ 1,91	21,34 $\pm$ 2,92	30,11 $\pm$ 2,57	29,29 $\pm$ 3,36	22,39 $\pm$ 4,18
n-6	2,45 $\pm$ 0,15	1,60 $\pm$ 0,11	1,78 $\pm$ 0,10	2,43 $\pm$ 0,18	2,35 $\pm$ 0,21	1,94 $\pm$ 0,23	2,24 $\pm$ 0,03
n-3	23,33 $\pm$ 2,26	9,65 $\pm$ 1,62	11,10 $\pm$ 1,87	18,22 $\pm$ 2,80	26,80 $\pm$ 2,57	26,31 $\pm$ 3,41	19,44 $\pm$ 4,16
n-6/n-3	0,11 $\pm$ 0,01	0,17 $\pm$ 0,03	0,16 $\pm$ 0,03	0,14 $\pm$ 0,02	0,09 $\pm$ 0,01	0,08 $\pm$ 0,01	0,12 $\pm$ 0,02

**Table 7.**

Top 3 list of marine species (mu: muscle, st: soft tissue, bl: blubber) based on Lipid Quality Components (LQCs) (A) and contaminant corrected LQCs (B: LQC/Hg; C :LQC/PCB).

LQCs	(A) Species	Tissue	LQC <sup>1</sup>	(B) Species	Tissue	LQC <sup>1</sup>	LQC <sup>1</sup> /Hg <sup>2</sup>	(C) Species	Tissue	LQC <sup>1</sup>	LQC <sup>1</sup> /P CB <sup>2</sup>
LA	Little auk <sup>3</sup>	mu	3,7	Ringed seal	bl	1,1	32801	Arctic char	mu	1,5	2200
	Brünnichs guillemot	mu	3,2	Hooded seal	bl	1,6	17696	Capelin	mu	1,1	2128
	Common eider	mu	3,0	Harp seal	bl	1,3	15028	Atlantic salmon	mu	1,3	1944
LNA	Blue mussel	st	1,2	Ringed seal	bl	0,4	12252	Arctic char	mu	0,9	1328
	Little auk <sup>3</sup>	mu	1,0	Hooded seal	bl	0,8	8864	Atlantic salmon	mu	0,7	1069
	Arctic char	mu	0,9	Harp seal	bl	0,5	5567	Capelin	mu	0,5	864
AA	Common eider	mu	10,5	Ringed seal	bl	0,4	11437	King eider	mu	9,1	5081
	King eider	mu	9,1	Walrus	bl	0,7	6245	Common eider	mu	10,5	2637
	Walrus	mu	6,1	Harp seal	bl	0,5	5554	Greenland cod	mu	2,6	1973
EPA	Snow crab	mu	32,3	Ringed seal	bl	9,0	268719	Capelin	mu	14,0	26606
	Iceland scallop	mu	23,4	Hooded seal	bl	7,0	77274	Northern shrimp	mu	22,0	25945
	Northern shrimp	mu	22,1	Walrus	bl	8,5	76227	Iceland scallop	mu	23,4	23541
DHA	Atlantic cod	mu	34,5	Ringed seal	bl	9,0	268901	Atlantic cod	mu	34,4	30195
	Greenland cod	mu	34,1	Hooded seal	bl	10,5	116342	Capelin	mu	15,0	28580
	Haddock	mu	29,6	Harp seal	bl	7,1	80173	Greenland cod	mu	34,1	26290
PRI+PHY	Little auk <sup>3</sup>	mu	1,2	Harp seal	bl	0,6	6341	Capelin	mu	1,1	2128
	Capelin	mu	1,1	Ringed seal	bl	0,2	5726	Arctic char	mu	0,5	748
	Spottet wolffish	mu	0,9	Hooded seal	bl	0,4	3952	Golden redfish	mu	0,7	661
SFA	Ringed seal	bl	11,7	Ringed seal	bl	11,7	348473	Capelin	mu	28,0	53473
	Beluga	bl	15,4	Harp seal	bl	18,5	208888	Arctic char	mu	23,7	35475
	Walrus	bl	15,5	Hooded seal	bl	15,6	172539	Atlantic salmon	mu	23,1	33890
MUFA	Narwhal	bl	70,3	Ringed seal	bl	57,1	1706334	Capelin	mu	35,5	67693
	Beluga	bl	68,2	Harp seal	bl	58,1	655593	Atlantic salmon	mu	43,5	63771
	Greenland halibut	mu	61,0	Hooded seal	bl	52,1	574555	Arctic char	mu	37,2	55716
PUFA	Iceland scallop	mu	59,3	Ringed seal	bl	29,3	874914	Capelin	mu	35,0	66827
	Greenland cod	mu	57,9	Hooded seal	bl	30,1	332231	Iceland scallop	mu	59,3	59584
	Atlantic cod	mu	57,9	Harp seal	bl	21,3	240750	Arctic char	mu	37,3	55961
n-3 PUFA	Iceland scallop	mu	54,6	Ringed seal	bl	26,3	785690	Capelin	mu	32,5	61886
	Atlantic cod	mu	54,3	Hooded seal	bl	26,8	295676	Iceland scallop	mu	54,6	54934
	Greenland cod	mu	53,8	Walrus	bl	23,3	209227	Arctic char	mu	33,8	50622

1) mass% of total fatty acid

2) (mg/gram)/total lipid (mass%)

3) no contaminant data

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## **Dietary composition and health indicators in North Greenland in the 1970's and today**

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and Hansen JC.

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# Dietary composition and health indicators in north Greenland, in the 1970's and today

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## ABSTRACT

**Objectives:** High levels of n-3 fatty acids and other nutrients in traditional Inuit food appear to provide some protection against cardiovascular diseases and type 2 diabetes –diseases of affluent industrialized societies. A transition towards increased amounts of imported food will probably increase the occurrence of these diseases. However, since the 1970's it has become evident that the marine-based Inuit diet also contains high levels of potentially toxic lipophilic organic pollutants and heavy metals. Since these two opposing effects on health appear to be inseparable, the phenomenon has been known as “The Arctic Dilemma”. However, both the fatty acid composition and the contaminant levels vary in Greenlandic food items. Thus in principle it is possible to compose a diet where the benefits and risks are better balanced. Our objectives were to compare traditional and modern meals in Greenland regarding their dietary composition, nutrients, and health indicators.

**Study design:** The present study was a cross-sectional dietary survey as part of the Arctic Monitoring and Assessment Programme (AMAP)

**Methods:** Dietary components, fatty acids, and nutrients in 177 traditional meals collected by duplicate portion method in Uummannaq municipality, north Greenland in 1976 were compared with 90 duplicate meals sampled in Uummannaq town in 2004. Anthropometric

measures (weight, height, and body mass index, BMI) and blood lipids were measured as health indicators among the participants.

**Results:** Between the traditional food collected 30 years ago and the food from 2004, significant differences were found in the dietary composition. The percentage of local food had decreased, and with it the content of n-3 fatty acids. Also, the intake of many vitamins and minerals had decreased, and was below Nordic Nutrient Recommendations in 2004. Vitamin A, B<sub>1</sub>, (B<sub>2</sub>), B<sub>12</sub>, iron, iodine, phosphorus, and selenium were correlated with n-3 content, whereas vitamin C, folate, and calcium were inversely correlated with n-3 but still low. The best balance between these two tendencies was found for medium intakes of n-3 (3-8 grams /day), corresponding to 20-40 % local food. Body weight, height, body mass index (BMI), cholesterol, and S-triglycerides had increased significantly.

**Conclusion:** The dietary changes to a more western fare were found to be negative resulting in less adequate nutrient coverage. However, we recommend not to increase the consumption of local products beyond the present level but rather to improve the quality of the imported food intake.

**Key Words:** Greenland, traditional and modern food, fatty acids and nutrients.

## INTRODUCTION

During the last 40-50 years the Greenlandic diet has changed to include an increasing amount and variety of imported food. This change in diet has been paralleled by a more sedentary lifestyle and coincides with an increased average body weight and an increased risk of diabetes and ischemic heart disease, (IHD) (1). These diseases were previously practically unknown in Greenland (2).

In 1970, two Danish doctors, Bang and Dyerberg, went to north west Greenland to investigate the observed low rate of cardiovascular diseases among Inuits living on a traditional fare (2,3,4,5). They took blood samples and made dietary interviews and found that the population had lower blood cholesterol and triglycerides, higher HDL (high density lipoprotein), indicating at that time a lower risk of contracting atherosclerotic diseases than Danes or Inuits living in Denmark (3,4).

They found that the Inuit diet, consisting mainly of marine animals and fish, was rich in protein and fat and very low in carbohydrates. The dietary fat, which contained a high proportion of certain mono-unsaturated and n-3 polyunsaturated fatty acids (PUFA), was different in composition from that of a European diet. They hypothesised that this special composition of dietary fats from marine animals and fish protected the consumers against ischemic heart disease.

Their results (6) suggested biochemical pathways for n-3 PUFA as modifiers of cardiovascular risk markers, which inspired a new cas-

cade of research investigating the alleged positive physiological effects of long-chained n-3 PUFA. From their five Uummannaq expeditions, their studies of serum lipids and food composition in Greenlanders have become an important part of the understanding of thrombotic diseases (7). Many of the health-promoting effects and modifiers of morbidity, observed in epidemiological studies both in Greenland and elsewhere have subsequently been confirmed by experimental studies using n-3 PUFA as dietary supplements (8,9,10).

Since the 1970's it has also become evident that the marine-based Inuit diet, although in principle healthy, contains high concentrations of lipophilic organic pollutants and heavy metals, which are potentially toxic (11,12). As part of the ongoing Arctic Monitoring and Assessment Program (AMAP) we systematically studied the human exposure to contaminants in six districts in Greenland 1999-2003 (13) combined with questionnaire and dietary surveys (11,13). The lipophilic contaminants are strongly associated with certain n-3 fatty acids in animal and human tissue (13). Since the adverse health effects of contaminants and the beneficial effects of n-3 fatty acids appear to be inseparable, this phenomenon has been known as "The Arctic Dilemma".

In 1976 Bang and Dyerberg performed more detailed dietary study (7,14,15) in the settlement of Igdlorssuit, Uummannaq municipality, where fishing, whaling and seal-hunting were still the main provisions of dietary resources. The participants were 33 local Inuits who each provided blood samples for lipid analysis and collected 5-7 duplicate daily meals. The meals were subjected to macro nutrient- and fatty acid analysis. In addition, the participants made a dietary record of the same period. From this record the general nutrient contents of their diets were estimated. This part of the investigation has never been published.

The duplicate meals from 1976 still exist as freeze dried aliquots. Dietary and anthropometric raw data are also still available. This has made it possible to compare traditional food with present day meals regarding nutritional value and contaminant burden, as well as to compare some physical health indicators.

We therefore decided to perform the "Sixth Uummannaq Expedition" and to collect present day material in the same district and simulate the previous conditions and methods as closely as possible.

## **MATERIALS AND METHODS**

### **1976 meals and blood samples**

In May 1976, 17 women and 16 men (16 middle-aged married couples and one single women) were recruited by public invitation from Igdlorssuit, (now Illorsuit) Uummannaq district. Each person contributed 5-7 duplicate daily meals, a total of 177 food duplicates (solids only), and recorded their liquid intake. The food portions were

ground in a meat grinder, homogenized, frozen, and transported to Denmark for analysis of macronutrients and fatty acids. The remnants were later freeze-dried for storage. The participants also provided blood samples (after 12 hours of fasting). The meals and blood samples were analysed at Aalborg Hospital (Denmark) for lipid composition and by gas chromatography for fatty acid composition. The meals were further analysed for macronutrients, water, and ash content (14,15).

Regarding the composition of the duplicate meals, the participants completed a brief questionnaire and, aided by a trained dietician, made a dietary record (DR) covering three days of the food sampling period. Of the 33 participants 28 gave interviews covering a total of 73 days. The micronutrient content of the DR was calculated using a specially created database based upon Danish nutritional tables, (The Danish Food and Nutrition Board), supplemented with a few Greenlandic food items. Unfortunately this database no longer exists and therefore the nutrient content of the 2004 samples had to be determined by another method, namely chemical analysis, see below.

Anthropometric measures, weight and height were measured by a doctor using standardized hospital instruments, with the participants wearing only underwear. BMI was calculated as weight in kg divided by height in metres squared.

#### **2004 meals and blood samples**

Between 1976 and 2004, the population of the settlement Igdlorssuit (now Illorsuit) had decreased from 145 to 120 and to less than 20 in the relevant age group. We therefore decided to perform the study in Uummannaq town (population 1460). In May 2004, we collected duplicate meals, blood samples, and questionnaires from 30 age-matched Inuits, 15 married couples who were recruited by public invitation. The participants contributed a total of 90 daily food duplicates as above. Each day the food (solids only) was brought to Uummannaq hospital, the contents were laid out, the ingredients were identified /described, and weighed. The food items from each person were mixed, homogenized, and frozen at -20 degree C, and transported to Denmark for further analysis. Liquid intake was registered on a separate scheme. The participants gave blood samples for analysis of cholesterol, triglycerides, fatty acids, metals, and organic contaminants. Fatty acids (FA) were analysed at Lipid Analytical Laboratory, Guelph, Canada (13). The participants also answered a general questionnaire that included a 60-item semi quantitative food frequency questionnaire (FFQ), which was used to estimate the habitual intake. Danish Standard portion sizes were used to estimate the weight of the intake, except for local meat and fish, for which the portion sizes were set 30% higher. Since no comprehensive nutrient tables exist today for Greenlandic food items, it was not possible to make data-based micronutrient calculations of the 2004 FFQ. A provisional estimate of macronutrients was made from a semi quantitative FFQ for 4 districts in Greenland, using Danish and Canadian food tables (1).

The meals were analysed for macro and micronutrients by an accredited laboratory ("Eurofins" Kolding, Denmark). The sugar and vitamins from the recorded beverage intake (mainly vitamin C from orange juice) were obtained from nutrient tables and included later.

Metal content was analysed at DMU, (National Environmental Research Institute (Dept of Arctic Environment)), by FIAS-method. Fatty acids (FA) were analysed at DTU, (Technical University of Denmark (Biochemistry and Nutrition Group)) by gas chromatography. Prior to FA-analysis total lipids were extracted by Folch-method (16), and fatty acid methyl esters were prepared using a modification of Morrison and Smith (17).

Anthropometric measures, weight and height were measured by a doctor using standardized hospital instruments with the participants wearing only underwear. BMI was calculated as weight in kg divided by height in metres squared.

### **Statistics**

All the available raw data from the 1976 study were entered in SPSS statistics program 13.0 together with the 2004 results and the two sets of results were analysed separately and together when possible. Mean values of macronutrients and fatty acids from the two sampling year were compared by "Independent Samples T-test". Bivariate correlation analysis (Spearman) was performed between food content of local food and n-3 FA and between n-3 FA in food and plasma.

The participants were categorized into five groups based upon daily n-3 FA intake from duplicate portions (group 1:<3 g/day, group 2:3-4.5 g/d, group 3: 4.5-8 g/d, group 4: 8-13.8 g/d and group 5: above 13.8 g/d). Group 1 comprised only participants from 2004 and group 5 only participants from 1976.

## **RESULTS**

### **1976 meals**

The food composition obtained by dietary record (of the DP) contained fewer types of food items (table 1) than in 2004 (see below). Several participants included only 5-10 different items in their diets. The most extreme example was a 3-day diet only consisting of just seal meat, fruit juice-mix, and tea with sugar. The absolutely dominating ingredient was seal meat followed by Greenland halibut and other local fish. The variety of Danish products was also more limited in 1976 than it had become in 2004. For example only white bread and no dark bread was eaten; pasta was absent, fresh fruit was almost totally absent. Onion intake was common, but other vegetables and potatoes were seldom consumed. A few persons had a high intake of light beer (0.5 % alcohol), up to 5 litres a day. Energy from alcohol intake was not included in the total energy. The mean daily intake of

macronutrients and total energy are compared in table 2. In 1976 the energy intake estimated by chemical analysis of the DP was 6311 KJ, which was 19% lower than the estimation by diet record (7776 KJ).

### **2004 meals**

By the “duplicate portion method” (DP), a total of about 40 different ingredients could be identified in the 90 portions, but for each person the number of consumed item types was smaller (10 to 15).

The Greenlandic food items mainly consisted of seal meat, seal blubber, dried narwhal meat, narwhal skin (muktuk), Greenland halibut, catfish, capelin, and arctic cod (table 1). The Danish food items were mainly white and dark bread, pork and beef, rice, pasta, sugar, cheese, salami, and ship’s biscuits. The duplicate meals contained very little fresh fruit, potatoes, and vegetables, which was consistent with the supply available in the local store at this time of the year. Liquid intake consisted mainly of water, coffee or tea with sugar, sweetened fruit juice, and beer. Consumption of milk was not common, but two persons consumed a considerable amount of milk powder with their tea or coffee. The sugar content of the liquid intake was included in the energy calculations. Contribution from alcoholic drinks was not included since the reporting was considered unrealistically low. The relative content of Greenlandic food by solid weight was 23 %.

Dietary intake measured by a semi quantitative food frequency questionnaire (FFQ) covered the habitual intake over a period of several months, thereby minimizing seasonal variations (table 1).

The total energy intake in 2004 was 6569 KJ from DP (table2). However, alcohol was not included. Based upon a previous country-wide FFQ among the same age group, corrected according to Greenland import statistics, an average alcohol intake has been estimated to 14 E% (1). Thus the total energy intake is probably an under-estimation of the real intake.

The relative weight % of Greenlandic food (23.3%) was the same as obtained by semi quantitative food frequency questionnaire (22.7%). A provisional estimate of the total energy by FFQ was 6068 KJ using Canadian and Danish food composition tables (1) and the E% of Greenlandic food was 20.7.

The mean daily intakes of vitamins and minerals (table 3) were calculated per 10 MJ to compare with Nordic Nutrient Recommendations (NNR 1996). In general, the micro nutrient intakes of the traditional meals from 1976 were much higher than of the modern meals from 2004, except for vitamin C and folate, which were very low for both years. The vitamin C intake had increased slightly in 2004, but the median intake was still low (12.5 mg). This means that for almost 50% of the participants the intake was lower than 10 mg/day, which is considered the scurvy limit. Of the mean vitamin C intake 55% was contributed by orange juice. Only four persons (2004) had a vitamin-

C intake meeting the recommendations. Two of these persons had a regular consumption of orange juice. However, the other two did not consume fruits and vegetables to explain their vitamin C intake, but they both had a very high consumption of milk powder. (Vitamin C is added to milk powder products sold in Greenland.) The fibre content was extremely low in 1976, with a mean of 1.4 g/day. With more potatoes and bread in the diet, this ingredient had increased in 2004 to a calculated mean of 10.5 g/day (table 2). However, the NNR requirement of 20-30 gram fibre per day was still not met.

The vitamin-A intake was high and almost exclusively of animal origin. Beta-carotene intake was consequently below 10% of total vitamin A. Thus in general the antioxidant content of the diet was low except for Se, which was high. This meant that among 75 % of the participants (1976) the Se intake was above the "individual maximum level for safe daily intake" (18). Calcium was low for both years, and high only among the very few persons who consumed milk or milk powder, but the other minerals were sufficient or high (iodine and iron).

Table 4 shows the fatty acid composition (% of total fatty acids) in duplicate meals and plasma phospholipids. Since n-3 fatty acids occur in high concentration in food of marine origin, the dietary differences were also manifested in the fatty acid profiles of both the diet and of the plasma phospholipids (table 4). In 1976, the food had a very high relative content of n-3 fatty acids, resulting in a mean daily consumption of n-3 fatty acids of 8.5 grams and an n-3/n-6 ratio of 3.3. The resulting plasma n-3/n-6 ratio was 1.7. In the 2004 group, significant differences were found both in the dietary and plasma lipid patterns. All the n-3 FA's had decreased significantly and linoleic acid (C18:2,n-6) had increased. The n-3/n-6 ratio in food (now 0.87) was 26% of the 1976 value and the ratio in plasma (now 0.60) was 35% of the 1976 value. The n-3 content of the food is a very strong indicator of local food content,  $r=0.7$ ,  $p<0.0001$ . Although the FA's of the plasma phospholipids represent more long-term eating habits, there was still a very good correlation between duplicate meal n-3 and plasma phospholipid n-3,  $p<0.0001$ .

The categorization of participants into five groups of n-3 intake shows that intakes of vitamin C, folate, and Ca were below NNR and decreased with local food content (Table 5), whereas intakes of vitamin B<sub>12</sub>, Fe, P, and Se increased with local food content and were sufficient within all groups, Vitamin A, B<sub>1</sub>, and (B<sub>2</sub>) were below NNR in the lowest groups but sufficient in the higher groups.

Also the anthropometric findings differed significantly between the two sampling years, table 6. In 2004 the mean height among men had increased by 4 cm and the weight by 18.5 kg. Among women the height had not increased, but the weight had increased by 10 kg. The resulting increases in BMI, in 2004 categorize 53% of men and 13% of women as obese (BMI>30), whereas in 1976 no obese persons were found in either sex group.



## DISCUSSION

During the last 30 years, substantial changes have taken place in Greenland, and the proportion of the population living in smaller settlements has decreased by more than 30 %. This represents a shift into larger settlements and towns, where the food supply is different and broader.

Thus the study performed in 1976 concerns the about 27% of the population living in settlements, whereas the study performed in 2004 represents the 82 % of the population who live in towns. A comparison of these two situations can therefore not just be used as expression of a temporal trend, since it is also a comparison between a settlement and a town, where the settlement depends more on traditional food supply. But taken together the two studies can be used to illustrate how the relative composition of local and imported food, indicated by n-3 FA content, affects the dietary nutrient content and other health indicators.

Since no up-to-date, comprehensive nutrient data base exists for Greenlandic food, to estimate daily nutrient intake in the 2004 samples we had to rely on a direct chemical analysis of the food collected by the “duplicate portion method” (DP). This method gives a precise analysis of the nutrient content in the collected food, and can thereby be used to characterize the sufficiency of different dietary compositions, e.g. different proportions of local and imported food, even if these are not representative for each participant’s more long-term eating habits.

In comparison to this method, various dietary recall or registration methods have other deficiencies and advantages. They rely on the participants’ memory, ability to evaluate portion sizes, and recall bias. In addition, calculation of nutrient content depends on using a database which may not be accurate for all food items or nutrients. A data-based calculation of nutrients from a dietary record of a DP tends to give higher nutrient values than a corresponding chemical analysis of the DP. However, the DP is likely to underestimate total energy intake (19). Therefore comparisons should be regarded with caution and at best based on energy adjusted intakes (19). Since dietary recalls e.g. food frequency questionnaires give more representative pictures of long-term eating habits we used these to supplement the other methods.

In general, the duplicate portion method and the food frequency questionnaire, FFQ, 2004 agreed on the relative content of most items, especially concerning the average percentage of Greenlandic food and major items of Greenlandic or Danish products. Differences were found for potatoes, fruit, tomatoes, and pepper fruit, for which the FFQ gave higher intakes. This can be explained by the time of year for the sampling of the duplicate portions. The sampling took place in May when the first supply ship had not yet arrived and the local store was out of potatoes, fresh fruit, and vegetables. Compared with the DP, the FFQ covered a longer period of the year. One can there-

fore consider the DP to be representative for the sampling time but not for the entire year.

In 1976 the 3-day diet record and DP covered the same time period as in 2004 before the ice broke, and therefore approximately the same supply situation.

Micro nutrients were not measured by chemical analysis in the DP from 1976 and nutrients could not be calculated from the FFQ from 2004, since the previously used database no longer exists and no new database has been established. Therefore these were the best estimates under the present circumstances.

Thus as mentioned above the micronutrient content of the DR 1976 should be regarded with some caution, and comparisons should be based mainly on macronutrient contents of the DP's which were analysed directly for both samples.

However, the nutrient intakes were calculated in relation to the chemically analysed n-3 content in the DP's for both participant groups in the study thus adjusting for an appropriate biochemical dietary indicator in the food for better comparison. In addition, regarding the n-3 and n-6 fatty acids which were measured both in the food and in plasma phospholipids, the differences in dietary content between the two years were matched by differences in FA composition of plasma lipids.

### **Comparison of nutrient contents of 1976 and 2004 meals**

Both habitual and temporal changes in Northern Greenland have influenced the ingredient composition of the meals. In the traditional meals from 1976, almost 60% of the weight and 41 % of the energy came from Greenlandic products, whereas in the meals of 2004 only 23 % of the weight and (20-23 %) of the energy came from local products. The intake of seal meat in particular decreased from about 400 to about 60 grams per day, a decrease of 80%. Also, the consumption of local fish and birds had decreased significantly. The sugar intake was high both in 2004 and in 1976. In fact in 1976 the daily sugar intake constituted almost the same weight as the intake of bread.

A semi-quantitative FFQ in 1999-2003 (1) among 352 men and women in of the same age group gave the following energy percentages of macronutrients: fat 33.7 %, total carbohydrate 33.5%, sugar 14.8 %, protein 18.6 %, alcohol 14% and a relative content of Greenlandic food 21.3 E%. Thus for 2004 in general the two methods (DP and FFQ) gave consistent results except for the seasonal influences. The energy percentages found by DP and FFQ were consistent with a countrywide FFQ.

The levels of vitamins and minerals in the meals, which were well above the NNR in 1976, had decreased dramatically by 2004 except for calcium, which was unchanged low, and folate and vitamin C, which had increased. The low level of calcium can be explained by

the still prevailing low intake of milk products. The contribution from drinking water was not included. However, the drinking water in Greenland has a low mineral content, as it originates from melted ice or snow (20).

The increase in mean vitamin C intake can mainly be explained by increase in consumption of orange juice, high intake of vitamin C - enriched milk powder by a few persons and a minor general increase in consumption of fresh fruit and vegetables. The 400 % difference in folate intake is harder to explain. The best known sources of folate are liver and green vegetables. The intake of liver appears to have decreased and the intake of green vegetables has not increased. Bread and cheese are medium range sources of folate and they have both increased, which may explain part of the increase. An alternative explanation would be that the data base used in 1976 underestimated the folate content of the Greenlandic food items. In any case, both calcium, magnesium, vitamin C, E, and folate were found to be below recommendations, and public health measures should be taken to help ensure better provision of these nutrients. The low Ca intake may be influential on the high rate of post menopausal osteoporosis in Greenland (20,21). Although vitamin D was not measured in this study, other researchers have found vitamin D insufficiency in Greenlanders living on westernized fare (23).

Since many adult Greenlanders do not tolerate milk products well it does not seem to be a solution to recommend increased milk consumption (except cheeses) to improve Ca status. It is also relevant that the supply of milk products is not constant and prices are high. The enrichment of milk powder with vitamin C or Ca is a solution only for a small target group. It may therefore be a better solution to enrich certain other food products with Ca, for example flour, which in addition could be enriched with folate.

Even if the measured vitamin C intakes were far below the recommendations, scurvy or other deficiency symptoms have never been documented among Inuits and their actual vitamin C needs are not known. Thus at the present time we would not recommend dietary enrichment with vitamin C.

After categorizing the participants into 5 groups of n-3 fatty acid content (local food indicator) in the DP's, it appeared that n-3 content was a strong determinant of other nutrients. Vitamin A, B<sub>1</sub>, (B<sub>2</sub>), B<sub>12</sub>, Fe, P and Se increased with n-3 content whereas vitamin C, folate, and Ca decreased. The best balance between these two tendencies, would be found for medium intakes of n-3 (3-8 grams/day) corresponding to 20-40 % local food.

Nutritional coverage among Greenland Inuits, who have very high marine food intake, are hard to compare with dietary studies among other Arctic populations or even other Inuits. However, a comparison of Canadian Inuits (Nunavut) on days with traditional food versus days without traditional food (24) supports the nutrient profiles of the present study and points to the same nutrients being marginal.

Another Canadian study showed that young Inuits on more westernized fare had a 4-6 fold relative risk of vitamin A inadequacy compared with older Inuits eating more traditional food (25).

These nutritional considerations of the Greenland diet can, however, not stand alone. Several studies have measured high levels of organic pollutants and heavy metals in local Greenlandic food products (22) as well as in human plasma (13), due to this we cannot recommend to increase the consumption of Greenlandic products in general above the present level. However, both the fatty acid composition and the contaminant levels vary in Greenlandic food items, so in principle it should be possible to compose a diet in which the benefits and risks would be better balanced.

The levels of body weight, BMI, and cardiovascular risk factors found in 2004 are in agreement with recent results from 5 districts of Greenland including both settlements and towns (1). In a study performed in 1970 among 130 Inuits from west Greenland, Dyerberg et al (3) found no overweight persons among the participants. Both s-cholesterol and triglycerides were found to be significantly higher in 2004. The lower intake of n-3 and thereby lower plasma n-3 content is consistent with an increase in triglycerides. Furthermore, high BMI is a very strong determinant for adverse development in triglycerides and other cardiovascular risk indicators

The increase in body weight and possible adverse development of cardiovascular risk indicators are not sufficiently explained by the energy and nutrient composition of the diets. The energy or fat intakes have not increased, and sugar intake has not increased significantly. However, the more western lifestyle has most likely caused a decrease in physical activity level in Greenland (26) which may partly explain the increased BMI. The same paper also shows (26) that serum lipid cardiovascular risk factors exacerbate with westernisation.

## CONCLUSIONS

Based upon the dietary comparison between traditional food samples from 1976 and samples of a modern composition from 2004, and between the different groups of local food indicated by n-3 intake, our overall conclusion is that a diet with a high percentage of Greenlandic food items in general provides sufficient vitamin and mineral coverage. However, there are a few exceptions namely Ca, Mg, vitamin C, E, and folate. It also appears that a diet with an average percentage of Greenlandic food of 20-40% (the present country mean) will meet the NNR for most nutrients. In contrast to this, basing a diet exclusively on available Danish import products can be a risky situation in Greenland, since general availability and freshness is not always guaranteed. A diet with very high percentage of Greenlandic food imposes a strong risk of deficiency in Ca, vitamin C and folate and it also has a very low content of dietary fibre. We don't recommend to increase the consumption of Greenlandic products in general above the present level. If more fruit, vegetables, and potatoes were

added to the Greenland diet, the vitamin C, folate and dietary fibre intake would improve. Since increased milk intake is not an option, consumption of more bread especially Danish ryebread would improve the Ca intake.

Furthermore, it is important that the composition and quality of the imported products in the diet provide the best possible nutrient coverage, in particular that supplies of fresh or frozen fruit, vegetables and potatoes are ensured throughout the year.

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**Table 1.** List of daily intakes in grams, mean and (sd). Uummannaq 1976 dietary record (DR)  
<sup>a)</sup> compared with 2004 duplicate portions (DP) and food frequency questionnaire (FFQ).

Greenlandic products:	1976 (DR) n=28, 3 days	2004 (DP) n=30, 3 days	2004 (FFQ) n=30
Seal meat	410 (343)	55.6 (61)***	65.0***
Greenland Halibut	50.4 (64)	31.7 (45)	22.2*
Narwhal	0	11.1*(22)	29.0*
Ammasset (Capellin)	0	4.5 (15)	0.9
Seal liver	17.6 (49)	4.3 (9.5)	3.1
Muktuk	0	4.2 (9.8)*	7.1*
Other fish ( catfish, cod etc)	40.0 (64)	10.3 (23)*	11.0*
Seal blubber	9.2 (23)	3.1 (5.5)	2.8
Birds	16.2 (42)	0.4 (1.8)	2.7
<b>Danish products:</b>			
Bread	64 (44)	153 (56)***	125***
Sugar (total)	56.7 (27)	87.9 (49)	60.7
Beef and pork meat	18.9 (32)	58.9 (52)*	55.0*
Rice	34.4 (44)	40.3 (60)	20.3
Potato	13.9 (29)	39.0 (46)	108*
Pasta	0	22.8 (37)	not asked
Cheese	1.4 (4.4)	19.3 (28)*	20.3*
Cakes, cookies, chocolate	16.4 (31)	19.8 (23)	5.5
Milk	28.0 (60) whole milk	17.0 (54) milk powder 150 whole milk equivalent	104 whole milk
Salami	11.6 (22)	10.7 (22)	7.8
Ships biscuits and crackers	17.7 (35)	6.4 (13)	not asked
Apples and pears	0	6.0 (13)	41.8
Butter and margarine	7.5 (7.4)	5.0 (6.5)	14.9
Chicken	3.9 (11)	4.5 (11)	7.0
Tropical fruit	4.8 (10)canned	4.0 (11) raw	34.0 raw or canned
Eggs	0	2.5 (6.5)*	10.4
Cooked vegetables	5 (14)	2.5 (7.6)	27
Tomato and pepper fruit	0	5.3 (19)	4.3
Onion	22.1 (33)	0.5 (5.7)*	not asked

a) DR covered 3 days of the 5-7 day collecting period of the DP

Significance levels are shown for independent samples T-test of 2004 versus 1976 p<0.05\*, p<0.01\*\*, p<0.001\*\*\*



**Table 2.** Daily intakes of energy and macronutrients, mean and (sd). Uummannaq 1976 traditional meals, chemical analysis of duplicate portions (DP), and dietary record of duplicate portions (DR), compared with modern meals 2004 (DP) and food frequency questionnaire (FFQ).

	1976 DP (n=33) (5-7 days) Mean (sd)	1976 DR (n=28) (3 days) Mean (sd)	2004 DP (n=30) (3 days) Mean (sd)	2004 FFQ (n=30) Mean
Total energy KJ a)	6311(2602)	7776 (2629)	6569 (2398)	6068 b)
fat E%	40.0 (7.3)	29.3 (11.8)	35.1 (8.6)	
carbohydrate E%	36.3 (9.8)	40.4 (13.0)	49.0 (10.5)	
Protein E%	23.2 (5.2)	30.3 (12.0)	16.7 (4.1)	
sugar E%	not measured	12.4 (5.9)	16.7 (7.0)	
Alcohol E%	not measured	3.0 (10) Not included	not measured	
dry matter gram	277 (80)	not measured	262 (95)	
Dietary fibre c)		1.4 (0.8)	10.5 (5)	
Greenlandic food % solid weight		59.5 (20)	23.3 (16.5)a)	22.7 (11.2)
Greenlandic food E%		41.4 (21)		20.7 a)b)

a) Sugar but not alcohol is included from beverage intake.

b) Provisional calculation using mean food intakes (table 1) and Danish and Canadian food tables.

**Table 3.** Daily intake of vitamins and minerals per 10 MJ, mean and (sd). Uummannaq 1976 by dietary record (DR)<sup>a)</sup>, compared with 2004 by duplicate portion method (DP)<sup>b)</sup> and Nordic Nutritional Recommendations (NNR 1996).

Nutrient	Uummannaq 1976 DR n=28, 3 days	Uummannaq 2004 DP n=30, 3 days	NNR 1996
Vitamin A (microgram)	2424(698)	1132 (1539)	1000
Vitamin B <sub>1</sub> (mg)	1.85 (0.5)	0.86 (0.31)	1.3
Vitamin B <sub>2</sub> (mg)	4.5 (2.6)	1.47 (0.75)	1.4
Vitamin B <sub>6</sub> (mg)	2.15 (0.6)	not analysed	1.3
Vitamin B <sub>12</sub> (microgram)	27.4 (28)	6.04 (4.0)	2
Niacin (mg)	92 (40)	not analysed	16
Folate (microgram)	67.5 (77)	262 (117)	360
Vitamin C (mg)	27.7 (40)	56 (78)	70
Vitamin D (microgram)	6.3 (10)	not analysed	6
Vitamin E (mg)	8.0 (2.2)	5.13 (2.3)	10
Calcium (mg)	587(281)	544 (285)	1100
Iron (mg)	117 (66)	31(20)	14-21
Magnesium (mg)	335(51)	205 (61)	340
Phosphor (mg)	1620(338)	1393 (362)	850
Zink (mg)	117.0 (5.4)	10.4 (4)	11
Selenium (microgram)	242 b)	154 (62)	50

a) DR covered 3 days of the 5-7 day collecting period of the DP, the nutrients were calculated from a nutrient database

b) The nutrients were chemically analysed in duplicate portions

**Table 4.** Fatty acid (FA) content in % of total FA's in food (duplicate portions) and plasma phospho lipids. Uummannaq 1976 and 2004, compared by independent samples t-test <sup>a)</sup>.

% of lipids in food	1976: mean (sd) (n=33, each 5-7 DPs)	2004 :mean (sd) (n=30,each 3 DPs)
mono-unsaturated FA	57.5 (5.3)	43.8 *** (5.6)
poly-unsaturated FA	19.8 (2.5)	14.2 *** (3.1)
saturated FA	22.7 (4.8)	39.3*** (7.8)
C.18:2,n-6	4.8 (2.5)	7.4 *** (3.2)
C.20:4,n-6	0.35 (0.2)	0.29 (0.09)
C.20:5,n-3	4.6 (1.2)	1.4*** (1.2)
C.22:5,n-3	2.6 (0.9)	0.7*** (0.7)
C.22:6,n-3	6.0 (1.4)	2.0*** (1.8)
N-3/n-6	3.3 (1.9)	0.88*** (0.8)
N-3 gram/day	8.5 (4.9)	3.8 *** (3.4)
N-6 gram/day	3.3 (2.5)	4.7 *** (2.0)
<b>% of plasma phospho-lipids</b>	<b>(n=33)</b>	<b>(n=30)</b>
mono-unsaturated FA	20.1(3.5)	17.8** (1.5)
poly-unsaturated FA	34.6 (7.0)	35.7 (1.9)
saturated FA	41.3 (7.6)	45.0 p=0.07(7.7)
C.18:2,n-6	8.7 (4.0)	15.3*** (5.1)
C.20:4,n-6	5.6 (1.5)	5.5 (1.2)
C.20:5,n-3	11.0 (3.5)	4.5*** (2.8)
C.22:5,n-3	not detected	1.3 (0.6)
C.22:6,n-3	11.1 (3.5)	6.4*** (1.6)
N-3/n-6	1.7 (0.7)	0.6*** (0.3)

a) p<0.05\*, p<0.01\*\*, p<0.001\*\*\*.

**Table 5.** Nordic Nutrient Recommendations (NNR) and marginal vitamins and minerals <sup>a)</sup>, means and (SD) in duplicate portion meals from Ummannaq, listed by daily intakes of n-3 fatty acids as indicators of local food content.

n-3 g/day	Year	Vitamin A microgram/10MJ NNR:1000	Vitamin B <sub>1</sub> mg/10MJ NNR:1.3	Vitamin B <sub>2</sub> mg/10MJ NNR:1.4	Vitamin C mg/10MJ NNR:70	VitaminE microgram/10MJ NNR:10	folate microgram/10MJ NNR:360	Ca mg/10MJ NNR:1100
<3	2004 (N=15)	527(348)	0.93 (0.32)	1.41 (0.79)	63 (99)	5.13 (2.9)	261 (114)	527 (246)
3-4.5	1976 (N=9)	1031(361)	1.67 (0.31)	3.21 (0.46)	28 (43)	7.89 (2.1)	80 (98)	727 (304)
	2004 (N=6)	681(512)	0.74 (0.18)	1.23 (0.82)	38 (53)	5.28 (1.44)	262 (171)	553 (243)
4-5-8	1976 (N=8)	2236(3436)	2.06 (0.57)	5.43 (2.28)	16 (18)	8.50 (1.69)	54 (46)	618 (256)
	2004 (N=6)	1805(1524)	0.73 (0.23)	1.71 (0.35)	62 (65)	5.08 (2.0)	270 (76)	676 (415)
8-13.75	1976 (N=7)	3386(5040)	1.78 (0.4)	4.56 (3.33)	40 (63)	8.00 (2.38)	39 (26)	442 (213)
	2004 (N=3)	3713(3463)	1.01 (0.59)	1.75 (1.16)	47 (42)	4.93 (2.30)	254 (145)	341 (187)
>13.75	1976 (N=3)	4409(6935)	1.43 (0.71)	4.44 (4.84)	28 (15)	5.00 (2.64)	127 (143)	400 (187)

<sup>a)</sup> B<sub>12</sub>, Fe, P, and Se were above NNR for all groups

**Table 6.** Health indicators (mean and (S.E)) for participants of the dietary studies in Uum-mannaq 1976 and 2004 compared by independent samples t-test, men with men, and women with women<sup>a)</sup>.

	<b>1976, men ( n=11)</b>	<b>1976 women (n=17)</b>	<b>2004 Men (n=15)</b>	<b>2004 women (n=15)</b>
Age	46.7 (3.9)	40.6 (3.1)	50.5 (2.2) ns	46.7 (2.2) ns
height, m	1.66 (0.17)	1.56 (0.13)	1.70 (1.1) ns	1.57 (2.1) ns
weight, kg	68.2 (1.4)	57.5 (2.2)	86.7 (4.0)***	67.3 (2.4)***
BMI	24.7 (0.47)	23.6 (0.75)	29.7 (1.2)***	27.4 (1.4) ***
Overweight BMI (25-29.9)	28%	18%	26%	33%
Obese BMI >30.0	0%	0%	53%	13%
Cholesterol mmol/l. b)	5.03 (0.17)	5.03 (0.17)	5.83 (0.23)***	6.25 (0.30) ***
Triglycerides mmol/l	0.64 (0.06)	0.66 (0.05)	1.76 (0.39) ***	1.34 (0.50) ***
HDL			1.58 (0.40)	1.86 (0.18)

a)  $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$

b) from (Dyerberg et al 1977)

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## **Dietary composition and contaminants in North Greenland in the 1970's and 2004**

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# Dietary composition and contaminants in north Greenland, in the 1970's and 2004

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## ABSTRACT

**Objectives:** The fatty acid composition and other nutrients in traditional Inuit food appear to provide some protection against diseases of affluent industrialized societies, such as cardiovascular diseases and type 2 diabetes. A transition towards increased amounts of imported food might increase the occurrence of these diseases among Inuit. However, since the 1970's it has become evident that the marine-based Inuit diet also contains high levels of potentially toxic lipophilic organic pollutants and heavy metals. Since these two opposing effects on health appear to be inseparable, the phenomenon has become known as "The Arctic Dilemma". However, both the fatty acid composition and the contaminant levels vary in Greenlandic food items. Thus in theory it is possible to compose a diet where the benefits outweigh the risks. Our objective was to compare traditional and modern meals in Greenland regarding dietary composition, content of n-3 fatty acids and contaminants.

**Study design:** The present study was part of the Arctic Monitoring and Assessment Programme, AMAP, comparing the results of dietary composition and nutrients in 177 traditional meals collected in Uummannaq municipality, north Greenland in 1976 with 90 meals sampled in Uummannaq town in 2004 under similar conditions. Eleven pesticides, 14 PCB congeners, heavy metals, selenium, and fatty acids were analysed in meals and blood samples from the participants. Contaminant levels were compared between 1976 and 2004 after adjustment for n-3 fatty acids, indicating local food content.



**Results:** Between the traditional meals collected 30 years ago and the meals from 2004, dramatic and significant changes have occurred in the dietary composition. The percentage of local food has decreased, and with it the intake of n-3 fatty acids.

Calculated as daily intake, all but three contaminants had decreased significantly. However, this could be explained by the lower intake of local food. After adjustment for n-3 fatty acid content in the food, significant declines of concentration in the local food were evident only for PCB and lead, whereas for mercury, DDTs, and chlordanes the levels were unchanged, and for hexachlorobenzene, mirex, and toxaphenes the levels had increased significantly.

**Conclusion:** The consumption of locally produced food has decreased in Greenland during the last 30 years and this has led to a reduction in the daily intake of contaminants. However, the concentrations of contaminants in local food items have not decreased, except for PCB and Lead. Therefore, we recommend that the consumption of local products is not increased beyond the present level, until the level of contaminants is reduced to a safer level.

**Key Words:** Contaminants, fatty acids, Greenland, traditional and modern food.

## INTRODUCTION

Since the 1970's it has become evident that the marine-based Inuit diet, although in principle healthy, contains high concentrations of heavy metals (Hansen1988, Hansen et al1990) and organic pollutants which are potentially toxic (AMAP 1998, Van Oostdam et al 2004, Bjerregaard et al. 2001). Subcutaneous fat collected during laparotomies from Greenlanders in 1979 had almost double the concentration of PCB's and DDT's than fat from a corresponding group of Danes (Jensen and Clausen 1979). Studies in several arctic countries confirmed that the pollutant levels in Greenland were in fact the highest in the Arctic (Van Oostdam et al. 2004) much higher than in the Scandinavian countries.

In 1970, two Danish doctors, Bang and Dyerberg, went to north west Greenland to investigate the observed low rate of cardiovascular diseases among Inuits living on a traditional fare (Bang et al 1971, Dyerberg et al. 1975,1977, 1978). They found that the Inuit diet, consisting mainly of marine food was rich in protein and fat and very low in carbohydrates. The dietary fat, which contained a high proportion of long-chained mono-unsaturated and n-3 polyunsaturated fatty acids (PUFA), was different in composition from that of a European diet. They hypothesised that this special composition of dietary fats originating from marine animals and fish protected the consumers against ischemic heart disease. Their results suggested biochemical pathways for the n-3 PUFA's as modifiers of cardiovascular risk markers (Bang et al. 1971, Dyerberg et al. 1975,1977, Dyerberg 1986). Many of the health promoting effects which have been proposed from epidemiol-

ological studies both in Greenland and elsewhere have subsequently been confirmed by experimental studies using n-3 PUFA as dietary supplements (Dyerberg and Schmidt 1993, Deutch et al. 2000 a,b).

The lipophilic contaminants mentioned above are strongly associated with the marine animal fat which is a substantial part of the Inuit diet (Deutch et al 2004). Thereby they are also associated with the long-chained n-3 fatty acids in marine animal fatty tissue. Methylmercury is highly prevalent in meat and organs of the same animals.

Since the two opposing aspects/effects of the lipids and lipophilic contaminants in the Arctic diet appear to be inseparable, the phenomenon has been known as "The Arctic Dilemma" (Hansen 2000).

The Arctic Monitoring and Assessment Programme has systematically monitored organic pollutants and heavy metals in animals and humans since 1994 (AMAP 1998, Deutch et al. 2000, Deutch and Hansen 2003, Van Oostdam et al. 2004). The contaminants determined in this study were chosen in agreement with this programme for comparison with international results. Besides the AMAP studies, only very few older results exist for comparative studies.

Because of the adverse health effects of the organic pollutants, in order to register any temporal trend it is an important task today to continue to monitor their levels in animal tissue, in human dietary provisions, and in human tissue. The ultimate purpose is to provide health advice for the local arctic populations.

In 1976 a detailed dietary study was performed (Bang and Dyerberg 1981, Bang et al. 1980) in the settlement of Illorsuit (formerly Igdlorsuit), Uummannaq municipality, where fishing, whaling and seal-hunting were still the main dietary resources. The participants were 33 local Inuits, who each provided blood samples for lipid analysis and collected 5-7 duplicate daily food portions which were subjected to macro nutrient- and fatty acid analysis.

The duplicate meals from 1976 still exist as freeze dried aliquots, and questionnaire dietary- and anthropometric data are also still available. This has made it possible to compare these traditional food samples with present day meals regarding nutritional value and contaminant burden.

In May 2004, we collected duplicate meals, blood samples, and questionnaires from 30 age- and sex matched Inuits in the town of Uummannaq imitating the previous study conditions and methods as closely as possible. The main purposes of the investigation were to compare the composition of the meals from 1976 and today, and to analyse and compare the level of contaminants in the diets to investigate the relative contribution from local Greenlandic food.

## MATERIALS AND METHODS

### 1976 meals and blood samples

In May 1976 the adult population of Illorsuit, Uummannaq was invited to participate in a dietary study, 16 middle aged married Inuit couples and one single women volunteered and complied to contribute 5-7 duplicate daily meals, a total of 177 daily food duplicates (solids only), and gave blood samples. The participants completed a brief questionnaire and a 3-day record of the duplicate meal content to a trained dietician.

The contents of the food portions were identified, ground in a meat grinder, homogenized, frozen, and transported to Denmark for analysis. The meals and blood samples were analysed by gas chromatography at Aalborg Hospital, Denmark, for lipid content and fatty acid composition. The meals were further analysed for macronutrients, water and ashes (Bang and Dyerberg 1981, Bang et al. 1980). The meal remnants were later freeze-dried for storage and were used for determination of contaminants in the present project. The blood samples from 1976 no longer exist so it was not possible to measure human contaminant levels from that study group.

### 2004 meals and blood samples

Between 1976 and 2004, the population of the settlement Illorsuit had decreased from 145 to 120 and to less than 20 in the relevant age group. We therefore decided to perform the study in Uummannaq town (population 1460). The participants were recruited by public invitation aiming at the same age group as above. The first 15 married couples who volunteered, met the age criteria, and complied to contribute daily food duplicates as above, were included (a total of 90 portions were contributed). All the participants gave written informed consent.

Each day the food (solids only) was brought to Uummannaq hospital, the contents were laid out and the ingredients were identified /described and weighed. The food items from each person were mixed, homogenized, frozen at -20 degree C, and transported to Denmark for analysis.

The fatty acid composition in food samples was analysed by gas chromatography at the Technical University of Denmark (Biochemistry and Nutrition Group). The n-3 fatty acids reported on were the sum of C18:3,n-3, C20:5,n-5, C22:5,n-3, and C22:6,n-3, and the n-6 FA's the sum of C18:2,n-6 and C20:4,n-6. The general nutrient composition of the meals was analyzed at the accredited laboratory Eurofins, Kolding, Denmark.

The participants gave blood samples for analysis of cholesterol, triglycerides, fatty acids, metals and organic contaminants. The plasma fatty acids were analysed at the Lipid Analytical Laboratory, Guelph, Ontario, Canada (Deutch et al. 2004). The participants also completed

a general questionnaire and a semi-quantitative food frequency questionnaire. The dietary composition and nutrient content of the meals from 1976 and 2004 will be presented in detail elsewhere.

Both groups of food samples, the freeze-dried food samples from 1976 and the frozen food samples from 2004 (as well as blood samples from 2004) were analysed concurrently for the same contaminants. All together 45 elements were measured in food and blood samples. In this paper we only report on the priority contaminants, the heavy metals (Cd, Hg, Pb), and organic contaminants listed below.

The metal content was analysed at National Environmental Research Institute (Dept of Arctic Environment) by FIAS-method. The blood and food samples were dissolved under pressure in nitric acid in Teflon bombs in a microwave furnace. Subsequently they were diluted in milliQ water. Mercury was analysed by cold vapour atomic absorption in a Perkin Elmer flow injection system (Asmund and Cleemann 2000). The mercury content in both meals and blood samples was measured only as total mercury i.e. inorganic + methylmercury. Other elements were determined by ICP-MS in an Agilent 7500cs apparatus.

The organic pollutants chosen and the analytical methods used were in accordance with the AMAP, Human Health Assessment group (AMAP 1998, Van Oostdam et al. 2004). According to this agreement the following organic pollutants were determined in microgram per litre (plasma) or microgram per kg lipid (food samples) at the certified laboratory, Le Centre de Toxicologie, Sainte Foy, Quebec, Canada.

Aldrine, alpha-, gamma-, cisnona-, oxy-, and transnona-chlordanes, p,p'- DDE and p,p'-DDT, hexachlorobenzene, beta-hexachlorocyclohexane (beta-HCH), mirex, and 5 toxaphene parlars 26, 32, 50, 62, 69, the following 14 PCB congeners (CB28, CB52, CB99, CB101, CB105, CB118, CB128, CB138, CB153, CB156, CB170, CB180, CB183, CB187). PCB Arochlor 1260 is reported for comparison with older studies ( $\text{PCB arochlor1260} = 5.2 \times \text{sum of CB138 and 153}$ ), Dieldrine is no longer measured. Aldrine, alpha- and gamma-chlordane and toxaphenes 32, 62, and 69 were all below detection limits and not reported further.

### Statistics

All the available raw data from the 1976 study were entered in SPSS statistics program 13.0 together with the 2004 results. Univariate descriptors were calculated for both years. The two population samples were compared by two tailed Independent samples T-test, and both population samples were analysed together by multiple linear regression analyses to identify predictors for contaminant levels in the food and human blood levels. Regarding predictors for human blood levels age, sex, and smoking were always independent variables.

The project was accepted by the Ethical Commission for Scientific Investigations in Greenland (KVUG).

## RESULTS

### Contaminants in human blood.

Table 1 shows the mean values (medians and SE) of blood or plasma concentrations of contaminants in Uummannaq compared with other districts in Greenland by the AMAP Project (Deutch and Hansen 2003, Deutch et al. 2004, ) and unpublished results. PCB and especially mercury guideline levels were exceeded by high percentages of the participants all over Greenland. Uummannaq had the highest levels of chlordanes, hexachlorobenzene, mercury, and lead, and the second highest PCB and DDT levels in Greenland. Regarding 2004 only men are shown for comparison with the two previous surveys, 1997 and 1999. The differences between Uummannaq 1997, 1999, and 2004 were not significant except for lead and selenium, and no trend was apparent. (In the 2004 blood samples women had significantly lower levels only for chlordanes). The very high selenium in 2004 may simply be due to recent consumption of Muktuk , whale skin. Since the blood or plasma samples from 1976 no longer exist we were not able to measure the contaminant levels in human tissue as part of the long range trend measurements.

### Comparison of meal composition of 1976 and 2004

In the traditional meals from 1976, almost 60% of the solid weight and 40 % of the energy came from local Greenlandic food items, whereas in the meals of 2004 only 23 % of the weight and (20-23 %) of the energy came from local products. The mean intake of seal meat in particular was lower, namely about 60 grams compared with 400 grams per day in 1976, a difference of 80%. Also different types of seals were eaten in 1976. Ringed seal (*Phoca hispida*) and Harp seal (*Phoca groenlandica*) were the most common but seven of the 28 participants reported eating larger seals such as Bearded seal (*Erignatus barbatus*) and Hooded seal (*Cystophora cristata*), whereas in 2004 only Harp seal and ringed seal were reported. The consumption of local fish and birds was also significantly lower in 2004. The mean composition of food intakes in 2004 was strongly influenced by the fact that the meals from about one third of the participants comprised less than 10% Greenlandic food.

The dietary differences were also manifested in the fatty acid profiles (% of total FA) of both the diet and of the participant plasma phospholipids. In 1976 the food had a very high relative content of n-3 fatty acids, resulting in a mean daily consumption of 8.5 grams of n-3 fatty acids with an n-3/n-6 ratio of 3.3. The resulting plasma n-3/n-6 ratio was 1.7. In 2004 all the n-3 FA's in food or plasma had decreased significantly and linoleic acid (C18:2,n-6) had increased. The mean daily intake of n-3 fatty acids was now 3.3 g and the n-3/n-6

ratio in food (now 0.87) was 26% of the 1976 value. The n-3/n-6 ratio in plasma (now 0.60) was 35% of the 1976 value.

### Daily contaminant intakes

The calculated mean daily contaminant intakes as microgram/day, and ng/Kjoule are shown in Table 2, and as microgram /kg body-weight /day in Table 3. The intakes of several organic pollutants (PCB, DDE, DDT) and heavy metals (Cd, Hg, Pb) as well as the trace element Se were significantly lower from the meals from 2004 than from the meals from 1976 (Tables 2 and 3). These differences can mainly, but not entirely, be explained by the lower mean percentage of local food in the 2004 meals. Men and women were tested separately and in 1976 the mean daily intakes in microgram were significantly higher in men, but not when calculated in ng/Kjoule or microgram/kg bodyweight per day. In 2004 the mean daily intakes were not significantly different in men and women.

A strong correlation was found between percentage of local food and the food content of n-3 FA,  $p < 0.0001$ . Thus n-3 FA content was used as an indicator of local food of marine origin. (The participants in the study did not consume any local terrestrial mammals or lake fish).

The participants were categorized into five groups based upon daily n-3 FA intake from the duplicate meals: (group1: below 3 g/d, group 2: 3-4.5 g/d, group 3: 4.5-8 g/d, group 4: 8-13.8 g/d, and group 5: above 13.8 g/d). Group 1 comprised only participants from 2004 and group 5 only participants from 1976. This categorization gave the results described below:

In all groups PCB intake was higher in 1976 (Figure 1). Multiple linear regression analysis showed that the correlation between PCB and the local food percentage was very strong,  $p < 0.001$ . Local food percentage, food content of n-3 FA, and sampling year were independent variables. The PCB content depended significantly on the sampling year, which was found to be an inverse predictor,  $r = -0.21$ ,  $p = 0.024$ . This means that the PCB level was lower in the local food items from 2004.

There was a small decrease in DDT's but the difference between 2004 and 1976 was not significant. Multiple linear regression analysis with the same variables as above showed that DDE was significantly correlated only with food n-3 content,  $p < 0.0001$  and that the effect of sampling year was not significant. This means, that the DDE content in local Greenlandic food items had not decreased significantly since 1976. However, DDT levels, which were very low compared to DDE levels, had decreased significantly.

The mean Beta-HCH was higher in all groups in 2004 and chlordanes and toxaphenes were higher in 3 out of 4 groups. Hexachlorobenzene was also significantly higher in all groups in 2004 (Figure 2). Thus for these compounds the mean daily intakes tended to be higher in the 2004 food (despite the lower local Greenlandic food content).

This indicates that the relative contamination levels in the local food products have actually increased.

Within groups mercury intake showed no significant difference between 1976 and 2004 but was strongly correlated with the n-3 group and percentage of local food. Multiple linear regression analysis confirmed the significant correlation with percentage of local food,  $p < 0.001$ . Hg was also significantly correlated with intake of narwhal,  $p < 0.001$ , seal meat,  $p < 0.001$  and seal liver,  $p < 0.0001$  after mutual adjustment. Thus, Hg content has not decreased in local Greenlandic food items.

Lead is the contaminant which has changed most, since the mean daily intake decreased to about 13% of the 1976 value. Thus the lead level in food strongly depended on sampling year. Furthermore, in the 1976 food samples Pb content was strongly positively associated with local food content,  $p < 0.0001$ , whereas in 2004, Pb did not depend on the percentage of local Greenlandic food in the diet. Birds shot with lead shot can contribute to human lead intake. But the food portions from 1976 and 2004 contained very little bird meat.

Cadmium, which had also decreased significantly, showed the same trend as lead, but less distinctly so.

Since the mean daily intakes of PCB, DDT's, Hg, Cd, and Pb had decreased in 2004 following the percentage of Greenlandic food items in the diet, there were also lower percentages of participants who exceeded the TDI values (microgram per kg body weight per day) and reference doses for these compounds (Table 4)(Health Canada 1996, WHO 1983, WHO 2003, US NRC 2000, Yang et al.1989).

Because the lipophilic contaminants are strongly associated with the fatty acids in the food, the content of each contaminant in the food was calculated per gram n-3 fatty acids (Table 5) thereby adjusting for marine food content. This calculation clearly shows, that in 2004, PCB was significantly lower and beta-HCH, hexachlorobenzene and mirex were significantly higher in the local food items, whereas DDE and mercury were unchanged.

The intakes of contaminants were all significantly correlated with intake of seal and whale but not with fish.

The plasma and blood levels of n-3 FA and contaminants in 2004 were also correlated with the local food percentage in the duplicate meals: (C20.5,n-3:  $r = 0.47$ ,  $p = 0.009$ , Hg:  $r = 0.47$ ,  $p = 0.01$ ). Significant correlations were found between daily intakes (microgram/day) and blood levels: (n-3/n-6:  $r = 0.67$ ,  $p < 0.01$ , C20.5,n-3:  $r = 0.70$ ,  $p < 0.001$ , Hg:  $r = 0.36$ ,  $p = 0.05$ ).

After adjusting for intake of local food, Hg in blood was significantly correlated with the reported intake of drinking water,  $r = 0.36$   $p = 0.027$ . However, in samples of the local drinking water Hg was below detection limit.

In contrast to mercury and n-3 FA, the calculated daily intakes of organic pollutants were not significantly correlated with the plasma levels of the pollutants among the participants (age, sex, and smoking were independent variables).

## DISCUSSION

There are different ways of changing the human dietary exposure to contaminants, one of which is to implement global measures to limit or discontinue production and use of the pertinent compounds. The production and use of PCB has been banned since 1979, the use of DDT has been limited since the 1970's by most countries (AMAP1998) and lead has not been added to petrol since the mid 1980's etc. Such measures will eventually lead to lower contaminant levels in food items. However, because of the persistent nature of many of these compounds they remain in the environment and are accumulated in the food web for an unknown length of time. Another way of changing human exposure is to change dietary habits, and this is already happening in the Arctic as part of the growing westernization. The mean relative dietary content of Greenlandic food (23%) is consistent with a countrywide population study (Deutch et al 2005) and other surveys (Pars 2000).

The calculated mean daily intakes of several organic pollutants (PCB, DDE, DDT), the heavy metals (Cd, Hg, Pb) and the trace element Se were found to be significantly lower in the meals from 2004 than in the meals from 1976. These differences can mainly, but not entirely, be explained by the lower percentage of local food in the modern meals.

The levels of selected organic and inorganic compounds in human tissue (blood and plasma levels) (Table 1) illustrate that Greenlanders are exposed to considerable pollution, that it is a widespread phenomenon and could be a serious health threat. Except for lead there is no evidence of significantly declining blood levels of any measured compound, within the period presented.

### Persistent organic pollutants

The possibility of comparing human tissue organic pollutant levels with older studies is limited to a measurement from 1979 (Jensen and Clausen 1979) in a rather small population sample of 17 autopsied Greenlanders (aged 22-45). Among these the lipid adjusted levels of PCB's and DDT's in subcutaneous fat were 5800 and 4700 microgram/kg lipid respectively. The PCB concentrations were in the middle range of the levels found in Greenland 1999-2003 (Deutch and Hansen 2003, Deutch et al 2004,,), but the sum of DDT levels four times higher.

As mentioned above, the calculated intake of PCB from 2004 meals was found to be lower than from 1976 meals. This was also the case after adjusting for percentage of local food and n-3 content. Table 5 shows clearly that the PCB/n-3 ratio is significantly lower in 2004



food. DDT has not declined significantly and beta-HCH, hexachlorobenzene and mirex have actually increased significantly. This supports the proposition, that the decrease in PCB is a true effect. However, it is still too early to consider this as a true environmental decline, since food choice (species, sex and age of catch animals) may confound the findings. Chlordanes, and toxaphenes have also increased but only borderline significantly.

### Mercury

The present study shows that mean dietary mercury intake has decreased along with the reduced intake of local food. The Hg intake was highly significantly correlated with percentage of local food in the diet and independent of the sampling year. Recent studies of mercury in Greenlandic animals also show no decreasing trend (Riget et al 2004).

In previous studies of human mercury exposure using blood samples collected in north Greenland (Uummannaq and Qaanaq) from 1981 to 1988, Hansen et al. (1990) found that mean mercury levels in blood of pregnant women increased from 33.7 microgram/litre to 45 microgram/l. They also found a linear association between blood levels and daily Hg intake estimated from relative intake of Greenlandic food. The mercury blood levels in north Greenland from 1999-2004 of more than 60 microgram/litre (table 1) appear to be a continuation of the increase observed in the 1980's. An increase in Hg blood level is not to be expected from the general lower intake of Greenlandic food and the present study estimates of mean daily dietary Hg intake, which have decreased since 1976 (from 84 to 34 microgram/day, Table 2). According to the equation used previously (Hansen 1988, Hansen et al 1990) a mean intake of 34 microgram per day would correspond to a blood level of only  $0.8 \times 34 = 27.2$  microgram/litre blood.

Therefore, there is an apparent contradiction between the lower dietary intake of Hg and the remaining high blood levels. A partial explanation of this is that the duplicate portion method may underestimate the total daily food intake (Willet 1998), but this would apply to the 1976 meals as well.

Other possible explanations are that besides the food, there are additional sources of human Hg exposures. Dental care has improved in Greenland, but also resulted in an increased number of amalgam fillings, which are known to contribute to human Hg exposure. However the magnitude of this contribution is generally below 0.25 microgram/l blood per dental filling (Luglie et al 2005).

Waste incineration plants are presently being installed in larger towns in Greenland. But like settlements and small and medium size towns in Greenland, Uummannaq still has uncontrolled burning of municipal waste at the outskirts of the town near the two drinking water lakes (700 m and 900 m respectively). With the growing use of industrial products in Greenland, more Hg is released from industrial and private waste to pollute the local environment. However, in

samples of the local drinking water Hg was below detection limits. Therefore the high prevalent Hg blood levels in northwest Greenland (Uummannaq and Qaanaq) remain unexplained.

### **Lead**

A significant decrease was found in estimated daily lead intake from food. Furthermore there was no correlation between food content of local products and lead concentration in 2004. In other words the local food was no longer a general source of lead. In studies in Uummannaq, northwest Greenland 1981-88, median Pb blood levels were 150 microgram/l (Hansen 1988). This means that by 1999 and 2004 there had been a significant decrease in blood levels of lead. This decrease can mainly be explained by the discontinued use of leaded petrol in the 1980's and lower atmospheric emissions (AMAP1998). However, it can not be excluded that the high blood lead levels in Uummannaq 1981 were influenced by the local Zink mine Maarmorilik, in use 1973-1990, which polluted the local fjord with lead and cadmium. Uummannaq and Illorsuit are both the same distance, 80 Km, from the Maarmorilik mine. The main sources of human lead exposure in Greenland today are tobacco smoking and lead shot used for bird hunting (Johansen et al 2004), lead shots are still being used in Greenland.

### **Cadmium**

There was a significant decrease in Cadmium intake between the traditional food from 1976 and the modern food from 2004, a difference that can mainly be explained by lower intake of Greenlandic food. Regarding Cd blood levels, these appear to have decreased from 1.8 microgram/l in the 1980's (Hansen 1988) to the recent values of 1.2 (1999) and 0.6 microgram/l (2004). This change may reflect the lower dietary intake of Cd, but since smoking is a major source of human Cd exposure the decrease could also reflect the fact that the number of smokers in Greenland is decreasing.

In summary, the intakes of both organic and inorganic contaminants from the Greenland diet are decreasing in parallel with the intake of local food. However, with the exception of PCB, lead, and possibly cadmium, the contaminant levels in the Greenland food items themselves have not decreased significantly. Therefore we recommend not to increase the general consumption of local food items unless less contaminated species e.g fish are chosen.

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**Table 1.** Concentrations of selected contaminants, organic pollutants in plasma and metals in whole blood. Uummanaq 2004, means, ( medians), and SE compared with our previous studies in Greenland<sup>a)</sup>.

Contaminant	Scoresby-sund, n=94	Tassiilaq, n=92	Sisimiut, n=94	Qaannaq n=75	Uumman-naq, n=10 men	Uumman-naq ,n=48 Men	Uumman-naq, n=15 men
Year	1999	2000-01	2002-03	2003	1997	1999	2004
PCB Arochlor 1260	113.4 (93.7) 8.7	35.6 (30.2) 2.4	12.4 (8.1) 1.3	39.5 (25.2) 3.9	32.2 (28.2) 5.5	51.9 (50.0) 2.9	39.1 (37.0) 6.2
Chlordanes	9.0 (7.1) 0.7	4.8 (4.0) 0.4	1.8 (1.1) 0.2	8.7 (4.7) 1.2	7.2 (6.0) 1.6	12.2 (11.7) 0.8	12.2 (12.7) 1.6
DDTs	13.3 (10.2) 1.0	9.8 (7.9) 0.7	3.1 (2.3) 0.4	7.5 (5.3) 0.9	12.2 (8.2) 3.3	13.0 (12.5)0.8	12.7 (10.7)2.5
Hexachloro-Benzene	1.8 (1.5) 0.1	1.1 (0.9) 0.07	0.7 (0.5) 0.06	1.8 (1.2) 0.2	2.3 (1.6) 0.5	3.5 (3.3) 0.2	3.1 (2.9) 0.5
Cadmium (Cd)	2.1 (2.0) 0.2	1.4 (1.3) 0.08	1.0 (0.7) 0.12	1.8 (1.4) 0.2		1.2 (1.1) 0.2	0.8 (0.8) 0.13
Mercury (Hg)	19.5 (16.3) 1.3	30.2 (25.8) 2.3	9.6 (7.3) 0.9	64.5 b) (52.3) 4.7		60.5 b) (58.4) 7.6	73.5 b) (59.0) 10.
Lead (Pb)	68.7 (61.5) 2.6	46.3 (39.7) 2.5	42.7 (36.5) 3.8	46.8 (39.0) 4.0		69.7 (58.4) 7.1	52.7 (47.0) 6.1
Selenium (Se)	225 (189) 13.5	189 (169) 7.2	173 (146) 16	584 c) (510) 42		488 d) (303) 67	847 e) (716) 115

a) randomly selected men and women (18-49 years) reference (15,17) and unpublished data.

b) US-EPA guidelines (4.2 microgram/litre) exceeded by 100% of the participants.

Percentage of participants with blood levels above guidelines (26) for maximum individual safe intake:

c) 40%, d) 25%, e) 66%.

**Table 2.** Daily intake of n-3 fatty acids, persistent organic pollutants and heavy metals, mean and (SE) from traditional Inuit food, Uummannaq 1976 (177 duplicate portions) and modern food, Uummannaq 2004 (90 duplicate portions) compared by two-tailed, Independent samples T-test .<sup>a)</sup>

	1976: Microgram per day	2004: Microgram per day	1976 ng/Kjoule	2004 ng/Kjoule
Sum of n-3 fatty acids (g/day)	8.5 (0.88)	3.8 (0.62)***	1.05 (0.1) Mg/Kjoule	0.56 (0.1)** mg/Kjoule
PCB Arochlor1260	36.79 (3.5)	12.05 (3.1)***	4.66 (0.5)	1.81 (0.5)***
beta HCH	0.29 (0.03)	0.56 (0.09)**	0.04 (0.004)	0.09(0.01)***
Chlordanes	9.36 (0.84)	7.23 (2.06)	1.15 (0.13)	1.08(0.29)
DDE	22.54 (2.1)	8.77 (2.98)***	2.86 (0.38)**	1.31 (0.44)**
DDT	7.83 (0.7)	1.35 (0.4)***	0.99 (0.13)	0.41 (0.14)***
DDE/DDT	2.88 (0.19)	6.47 (0.91)***		
Hexachlorobenzene	1.24 (0.13)	1.91 (0.51)	0.15 (0.02)	0.29 (0.07)
Mirex	0.184 (0.02)	0.31 (0.03)	0.023 (0.002)	0.039 (0.003)
Toxaphenes	4.23 (0.49)	4.08 (1.25)	0.54 (0.09)	0.64(0.18)
Cadmium (Cd)	69.23 (15.6)	17.54 (4.77)**	6.20(1.7)	2.41 (0.49)*
Mercury (Hg)	83.78 (14.58)	33.56 (8.23)**	8.66 (14.6)	4.99 (1.03)*
Lead (Pb)	38.14 (4.85)	5.13 (1.57)***	5.24(0.70)	0.73(0.20)***
Selenium(Se)	159.4 (18.7)	104.4 (11.2)*	17.7 (1.98)	15.5 (1.38)

a) p<0.05\*, p<0.01\*\*, p<0.001\*\*\*

**Table 3.** Daily intake per kg body weight of persistent organic pollutants, mean and (SE) from traditional Inuit food, Uummannaq 1976 (177 duplicate portions) and modern food, Uummannaq 2004 (90 duplicate portions) compared by two-tailed Independent samples T-test. <sup>a)</sup>

	1976: Microgram per kg body weight per day	2004: Microgram per kg body weight per day
PCB Arochlor1260	0.59 (0.053)	0.17 (0.053)***
beta HCH	0.005 (0.0005)	0.007 (0.0011)*
Chlordanes	0.15 (0.013)	0.10 (0.03)
DDE	0.36 (0.04)	0.12 (0.04)***
DDT	0.125 (0.01)	0.0185 (0.002)***
Hexachlorobenzene	0.019 (0.002)	0.026 (0.0075)
Mirex	0.002 (0.0003)	0.0044 (0.0004)
Toxaphenes	0.054 (0.01)	0.066 (0.017)
Cadmium (Cd)	0.80 (0.20)	0.22 (0.05)**
Mercury (Hg)	1.07 (0.17)	0.43 (0.10)**
Lead (Pb)	0.66 (0.11)	0.06 (0.017)***
Selenium(Se)	2.24 (0.28)	1.36 (0.14)**

a)  $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001$



**Table 4.** TDI values for contaminants and the percentage of participants with daily intakes exceeding the TDI values in Uummannaq 1976 and 2004.

	TDI: Microgram per kg body weight per day	1976	2004
PCB Arochlor1260	1.0 <sup>a)</sup>	10%	3.3%
beta HCH	0.3 <sup>a)</sup>	0%	0%
Chlordanes	0.05 <sup>a)</sup>	97%	40%
DDE	20 <sup>b)</sup>	0%	0%
DDT	20 <sup>b)</sup>	0%	0%
Cadmium (Cd)	1.0 <sup>d)</sup>	30%	3.3%
Mercury (Hg)	0.1 <sup>c)</sup> 0.23 <sup>d)</sup>	100% <sup>c) d)</sup>	60% <sup>c)</sup> 50% <sup>d)</sup>
Lead (Pb)	3.5 <sup>a)</sup>	0%	0%
Selenium(Se)	6.66 <sup>e)</sup>	0%	0%

a) Health Canada 1996.

b) WHO 1984

c) US EPA reference dose (RfD) (2000).

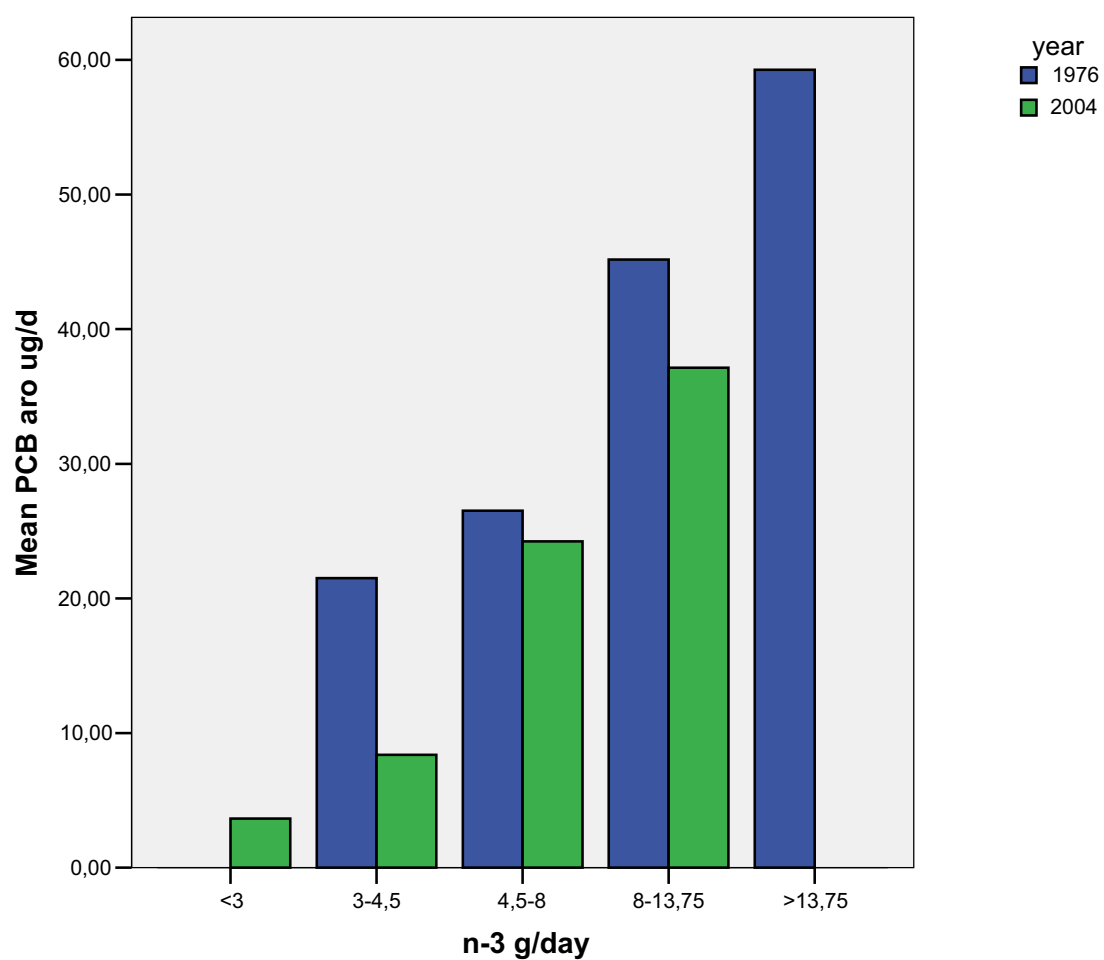
d) WHO/FAO 2003

e) Maximum safe daily intake corresponding to 400 microgram/day (Yang et al).

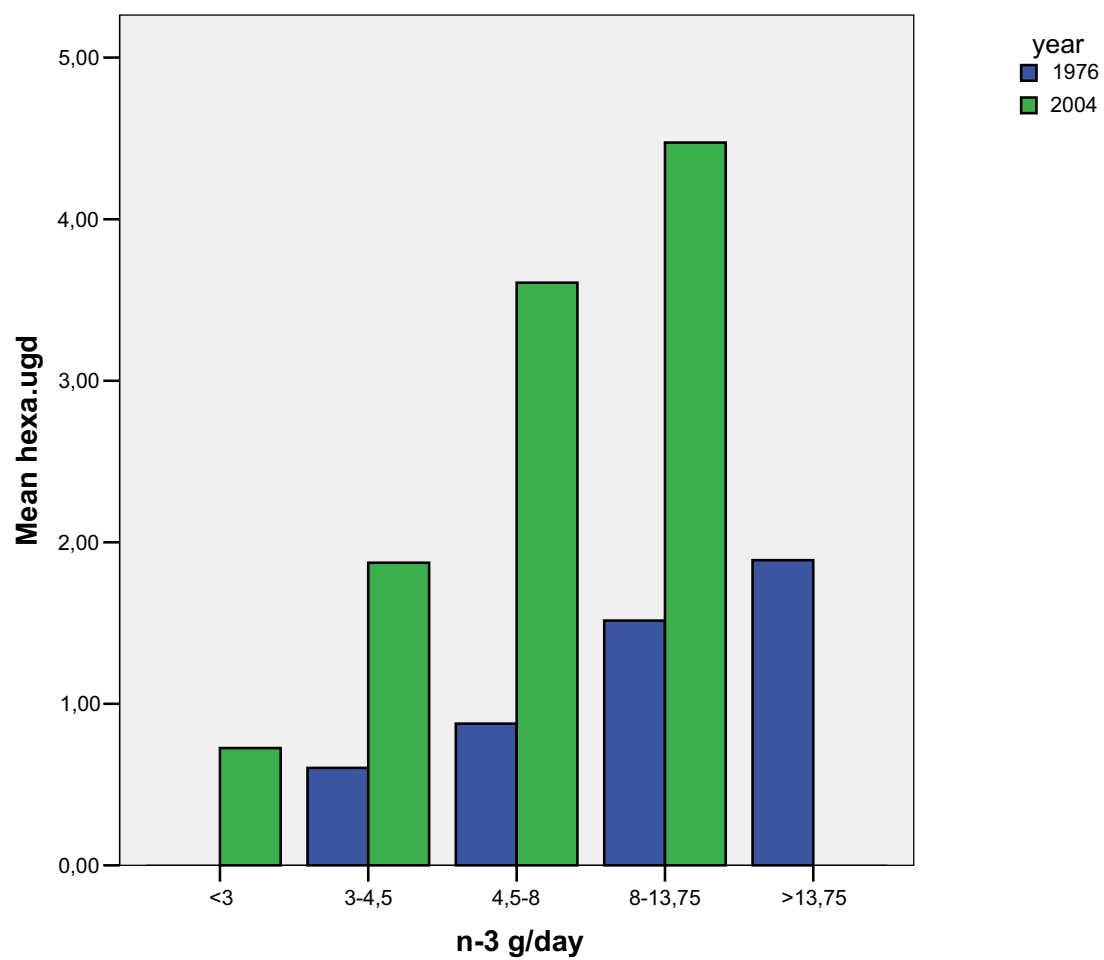
**Table 5.** Ratios between daily intakes of contaminants in micrograms and daily intake of n-3 FA in grams Uumannaq 1976 and 2004.

	1976	2004
PCB arochlor 1260	4.46	2.45 p=0.005**
DDE	2.68	2.01
beta HCH	0.037	0.26 p<0.0001***
Chlordanes	1.10	1.65
Hexachlorobenzene	0.14	0.48 p=0.001**
Mirex	0.022	0.082 p<0.001***
Toxaphenes	0.54	1.04 p=0.09
Mercury	9.26	9.47
Cadmium	7.28	9.68
Lead	5.29	1.85 p<0.001***

**Figure 1.** Daily intake of PCB arochlor 1260 , microgram/day, versus groups of daily intake of n-3 FAs.



**Figure 2.** Daily intake of hexachlorobenzene, microgram/day versus groups of daily intake of n-3 FA.



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**Impairment of cellular immunity in  
West Greenland sledge dogs (*Canis familiaris*)  
dietary exposed to polluted minke whale  
(*Balaenoptera acutorostrata*) blubber**

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# Impairment of Cellular Immunity in West Greenland Sledge Dogs (*Canis familiaris*) Dietary Exposed to Polluted Minke Whale (*Balaenoptera acutorostrata*) Blubber

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Minke whale (*Balaenoptera acutorostrata*) blubber is rich in organohalogen contaminants, mercury, and n-3 fatty acids. In the present study we show that a daily intake of 50–200 g of minke whale blubber causes an impairment of the nonspecific and specific cellular immune system in the West Greenland sledge dog (*Canis familiaris*). Immune reactions were measured by mitogen (PHA, Con A) and antigen (KLH) intradermal testing, and as the study used exposure levels similar to those of Inuits and polar bears (*Ursus maritimus*), it is reasonable to infer that Inuits and polar bears suffer from similar decreased resistance to diseases. It is speculated that food sources are depleted by thinning sea ice due to climate change and that more research should assess the forecasted rise in additive immunopathy effects in polar bears. Additionally, our study suggests that the fatty acid composition may be of importance when investigating combined immunotoxic effects of contaminated food resources in future Inuit and polar bear studies.

## Introduction

Polar bears (*Ursus maritimus*) from East Greenland, Svalbard, and the Western Russia are—due to their dietary intake of blubber from mainly ringed seal (*Phoca hispida*) and bearded seal (*Erignathus barbatus*)—the most organohalogen contaminant (OHC) polluted species in the Arctic (1–3). Pollutants in the Eastern part of the Arctic originate via airborne transportation from lower latitudes such as Eurasia and North America (1–3).

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Several studies have indicated that OHCs may have an impact on internal organs (4–6), the skeletal system (7), and hormones (8–12) in East Greenland and Svalbard polar bear populations. Little is known about the impact on the immune system. However, studies by Bernhoft et al. (13) and Lie et al. (14, 15) have indicated that both serum immunoglobulin G (IgG) level, humoral (antibody response following immunization), and cellular immunity (antigen and mitogen induced lymphocyte proliferation) may be impaired by OHCs in the Svalbard subpopulation of polar bears as well. This is in accordance with the general acceptance of the immune system being particularly sensitive to OHC exposure, as suggested by Tryphonas (16) and Vos and Luster (17).

In vivo studies of harbor seals (*Phoca vitulina*) fed contaminated Baltic fish showed that OHCs affect humoral (antibody response) and cell-mediated (lymphocyte proliferation) immunity (18–23). In free-ranging species, OHC immunotoxic effects, through mitogen-induced lymphocyte response and IgG concentration, have been suggested in bottlenose dolphins (*Tursiops truncatus*) (24), striped dolphins (*Stenella coeruleoalba*), and harbor seal (25), and in the St. Lawrence beluga whale (*Delphinapterus leucas*) (26–28). Similarly, a relationship between environmental organochlorine exposure and immunosuppression has been suggested for humans as well (29, 30).

To determine if immunotoxicity from OHCs in Svalbard and East Greenland polar bears is a true cause–effect relationship, we conducted a generational controlled study on domestic West Greenland sledge dogs (*Canis familiaris*). The domestic sledge dog was chosen as a phylogenetically relevant Arctic top predator for the polar bear. The exposed groups were fed dietary minke whale blubber rich in mercury, OHCs, and n-3 fatty acids while the control group was fed pork fat. The immune response after mitogen and antigen stimulation was measured using an intradermal test (IDT). The present study is a part of a larger study about health impacts from polluted minke whale blubber on the West Greenland sledge dog which is important for further understanding of the effects of environmental OHCs in exposed wildlife and humans.

## Experimental Section

**Experimental Design.** The present study is a part of a larger study about effects of contaminants in minke whale blubber in the West Greenland sledge dog. The animal experiments were performed on a license granted by the Home Government in Greenland. The experimental design was conducted as a randomized blind intervention study on West Greenland sledge dogs in Aasiaat, Disco Bay, West Greenland using real life exposure and taking seasonal fasting from yearly climatic oscillations into account. The parent generation (P) was composed of an exposed and a control group (8 bitches in each) and their 9 pups (4 exposed and 5 controls; all sisters and brothers) (Table 1). The exposed group was fed 50–200 g per day of organochlorine and mercury contaminated West Greenland minke whale (*Balaenoptera acutorostrata*) blubber rich in polyunsaturated lipids while the control group was fed pork fat (low in organochlorines, mercury, and polyunsaturated fatty acids) (Table 2). Bitches were fed exposed and control lipid source, respectively, immediately after entering the project at age two months, while pups were fed exposed and control lipid source, respectively, immediately after weaning. All dogs were kept in chains but exercised regularly, investigated routinely by a field veterinarian, and immunized for canine distemper virus, parvo virus, hepatitis virus, parainfluenza virus, and rabies. To minimize genetic



**TABLE 1. Data on the 16 Bitches (P Generation) and Their 9 Pups (F1 Generation) at Time of Intradermal Testing in the Present Study<sup>a</sup>**

individual	sex	age (weeks)	weight (kg)	group	mitogen/antigen <sup>b</sup>
bitch	F	49	29.6	exposed	PHA, Con A
bitch	F	49	30.0	exposed	PHA, Con A
bitch	F	64	22.5	exposed	PHA, Con A
bitch	F	23	17.8	exposed	PHA, Con A
bitch	F	27	28.7	exposed	PHA, Con A
bitch	F	41	24.0	exposed	PHA, Con A
bitch	F	60	26.9	exposed	PHA, Con A
bitch	F	60	21.3	exposed	PHA, Con A
bitch	F	49	29.2	control	PHA, Con A
bitch	F	49	30.4	control	PHA, Con A
bitch	F	64	23.3	control	PHA, Con A
bitch	F	23	19.4	control	PHA, Con A
bitch	F	27	24.4	control	PHA, Con A
bitch	F	41	26.7	control	PHA, Con A
bitch	F	60	29.8	control	PHA, Con A
bitch	F	60	22.7	control	PHA, Con A
puppy	M	18	16.3	exposed	PHA, Con A, KLH
puppy	F	18	16.0	exposed	PHA, Con A, KLH
puppy	M	18	15.9	exposed	PHA, Con A, KLH
puppy	M	18	15.3	exposed	PHA, Con A, KLH
puppy	M	21	18.4	control	PHA, Con A, KLH
puppy	M	21	17.4	control	PHA, Con A, KLH
puppy	M	21	15.4	control	PHA, Con A, KLH
puppy	F	21	14.4	control	PHA, Con A, KLH
puppy	F	21	20.3	control	PHA, Con A, KLH

<sup>a</sup> Bitches were followed up to 52 weeks and pups were followed up to 18 and 21 weeks. <sup>b</sup> PHA= Phytohaemagglutinin-P (mitogen); Con A= Concanavalin A (mitogen); KLH= Keyhole Limpet Hemocyanin (antigen; subcutaneous vaccination 4 weeks prior to dermal testing).

differences between the groups, all 16 bitches were paired sisters placed within each group. They were fed equal amounts of standardized Royal Canin Energy 4300/4800 dry dog pellets (50–200 g/day) to cover basic nutrients and microelements (<http://www.royalcanin.com/>) (Table 2). The aim of the immunizational trial was to study the cell-mediated immunity expressed via intradermal testing. Due to field logistics and the fact that bitches did not give birth at the same date, pups were intradermally tested at 18 (exposed) and 21 (controls) weeks of age. The P and F1 generations were followed for up to 52 and 21 weeks, respectively. It is noteworthy that the pups also may have received organochlorines and brominated flame retardants transplacentally and via milk in their first 8 weeks of life (31).

**Intradermal In Vivo Field Testing.** To characterize the effect of OHC exposure on the cell-mediated immunity, intradermal in vivo stimulation (delayed hypersensitivity; type IV) with mitogens (PHA and Con A; unspecific induced lymphocyte proliferation) was applied to the 16 bitches, and the same mitogens and an additional antigen (KLH; specific T lymphocyte response following immunization) was applied to the 9 pups (32). The intradermal test was applied to a 5 × 10 cm area laterally on the thorax. An area on the thorax without pigmentation or inflammation was trimmed using a No. 40 blade. Disinfecting agents, local anesthetics, or sedative were *not* used (Figure 1). Four weeks prior to the intradermal test, all pups were immunized (age span 14–17 weeks) subcutaneously with 1 mL of Keyhole Limpet Hemocyanin (KLH, number H7017, Sigma-Aldrich, Inc., St. Louis, MO) (1 mg KHL/mL in phosphate-buffered saline, PBS). The intradermal tests were performed by the same field veterinarian by application of 0.1 mL of Phytohaemagglutinin-P (PHA, Sigma-Aldrich) (50 µg PHA/mL and 200 µg PHA/mL in PBS solutions), Concanavalin A (Con A, type IV, Sigma-Aldrich) (50 µg Con A/mL and 250 µg Con A/mL in

PBS solutions), as mitogens, and KLH (20 µg KLH/mL and 200 µg KLH/mL in PBS solutions) as antigen (Table 1, Figure 1). Only the F1 generation was tested with KLH in two concentrations, and both generations were tested with PHA and Con A in two concentrations (Table 1, Figure 1). NaCl (0.1 mL 0.9%) was used as a negative control. Wheal size (diameter, height, and erythema) was measured at 24 and 48 h delayed type hypersensitivity (48 h reactions were excluded from the analyses as all these were magnitudes lower than the 24 h reaction) (Figure 2).

**Chemical Analyses.** Analyses of organochlorines and brominated flame retardants (Table 2) were conducted at The National Wildlife Research Centre, Carleton University (Raven Road), Ottawa, Canada according to Dietz et al. (33) and Luross et al. (34). Metals analyses were performed at the accredited (DANAK, DSyEN ISOyIEC 17025, No. 435) NERI-DAE Laboratory (Roskilde, Denmark; [www.neri.dk](http://www.neri.dk)) by flow injection mercury system (mercury), atomic absorption spectrometry (zinc and iron), and flow injection analysis system (selenium). Vitamin analyses (C and E) was conducted at the accredited (A7290) Eurofins Lab (Kolding, Denmark, [www.eurofins.dk](http://www.eurofins.dk)) using HPLC. Fatty acid analyses were conducted at DTU, Biochemistry and Nutrition Group (Lyngby, Denmark; [www.dtu.dk](http://www.dtu.dk)), using gas-chromatography and flame ionization (GC-FID). Prior to analysis total lipids were extracted according to Folch et al. (35) and fatty acid methyl esters (FAME) were prepared using the method by Morrison and Smith (36) with slight modification. The statistical analyses were performed with the SAS statistical software package (SAS V8 and enterprise guide V3.0, SAS Institute Inc., Cary, NC) and the level of significance was set to  $p \leq 0.05$ . As none of the data followed the normal distribution (Shapiro–Wilk test), the nonparametric Wilcoxon Two-Sample Test and Kruskal–Wallis Test were applied to analyze differences in wheal size (diameter; mm), height (0 to +4) and erythema and induration (0 to +3) between groups of bitches ( $n = 16$ ) and pups ( $n = 9$ ).

## Results

**Exposed vs Controls.** When pooling all exposed ( $n = 12$ ) and control ( $n = 13$ ) generations in the statistical analyses, exposed individuals responded more weakly than control individuals for *all* PHA and Con A intradermal reactions measured. Of the 24 PHA and Con A tests performed, 12 reactions (diameter of PHA-50 ppm and Con A-250 ppm; height and erythema of PHA-50 ppm) were significantly weaker (50%) in the exposed group (all  $p < 0.05$ ). In the most sensitive test (KLH in pups) all reactions (100%) were significantly weaker in exposed individuals (all  $p < 0.05$ ).

For Con A and PHA mitogens in the P generation, all intradermal reactions were weaker in the exposed group and 5 of 12 (42%) were statistically significant (Table 3). When comparing exposed and control P generations, the highest concentration of Con A and PHA was required in the exposed group to create their maximum amplitude intradermal reaction, and the increase was lowest in the exposed group (Table 3).

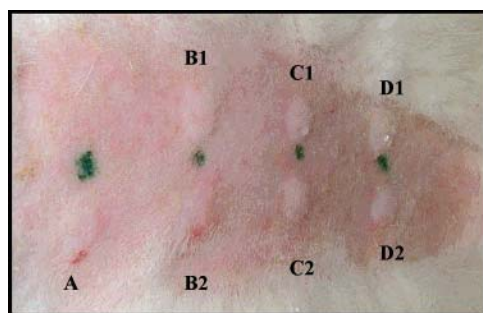
Exposed pups did not react intradermally to KLH at all. For Con A and PHA, 8 of 12 reactions were weaker in the exposed group (67%) and of these 1 (13%) was significantly weaker ( $p < 0.05$ ) (Table 3). In cases of low concentrations of PHA and Con A, *all* reactions were weaker in the exposed group and 1 significantly ( $p > 0.05$ ). As for bitches, the highest concentration of Con A and PHA was required in the exposed group to create their maximum amplitude intradermal reaction, and the increase was lowest in the exposed group (Table 3).

**Concentration and Age Differences.** When comparing the three different tests, the intradermal reactions were in most cases significantly stronger for Con A when compared

**TABLE 2. Pollutants and Nutrients in Minke Whale Blubber, Pork Fat, and Royal Canin (RC) Feed to Exposed and Control Bitches and Pups**

	minke whale blubber (n = 3 <sup>a</sup> )	pork fat (n = 3 <sup>a</sup> )	RC 4300 (n = 3 <sup>a</sup> )	RC 4800 (n = 3 <sup>a</sup> )
<b>pollutants (ng/g)<sup>b</sup></b>				
ΣPCB <sup>c</sup>	1716	<0.01	1.0	0.5
chlordanes	296	<0.01	<0.01	<0.01
ΣDDT	595	<0.01	<0.01	2.2
dieldrin	94	<0.01	<0.01	<0.01
ΣHCH	20	<0.01	<0.01	<0.01
HCB	28	<0.01	<0.01	<0.01
ΣPBDE <sup>d</sup>	63	<0.01	0.9	0.4
total mercury	0.005	0.001	0.001	0.002
<b>lipids (mg/g)</b>				
total lipid (mg/g)	571	954	196	263
n-3 fatty acids (mg/g)	64.30	8.56	4.53	4.08
% n-3 fatty acids of total fatty acids	17.5	1.3	3.4	2.5
<b>vitamins and microelements (mg/kg)</b>				
vitamin E	75	15	650	650
vitamin C	<10	<10	320	320
iron	<10	<10	250	250
zinc	0.6	0.1	190	190
selenium	0.15	<0.01	0.25	0.25

<sup>a</sup> The number of replicate analyses. <sup>b</sup> For BDE-99, concentrations detected in the method blanks were subtracted from the concentrations in the fed samples. <sup>c</sup> In the Energy 4300 and 4800 the ΣPCB concentrations consisted entirely of CB-153. <sup>d</sup> ΣPBDE concentration in the pork fat was 51% BDE-99, 28% BDE-153, and 21% BDE-183. ΣPBDE concentration in the Energy 4300 and 4800 was 54% BDE-47 and 46% BDE-99.



**FIGURE 1. Order of intradermal application of control, PHA, Con A, and KLH in a 21-week-old puppy from the control group at time = 0 using 1-mL, 27G, U-100 Insulin Myjectors (Terumo, Europe N. V., Leuven, Belgium). A, Negative control (0.9% NaCl); B1, PHA, 50 ppm; B2, PHA, 200 ppm; C1, Con A, 50 ppm; C2, Con A, 250 ppm; D1 = KLH, 20 ppm; D2 = KLH, 200 ppm. Green marks are for the orientation.**

with both PHA and KLH (all  $p < 0.05$ ). Such a difference was not found between PHA and KLH. Nearly all intradermal reactions were stronger in bitches than pups (Table 3) and for the diameter of PHA-50 ppm, PHA-200 ppm, and Con A-250 ppm in the controls, the difference was significant (all  $p < 0.05$ ) (Table 3).

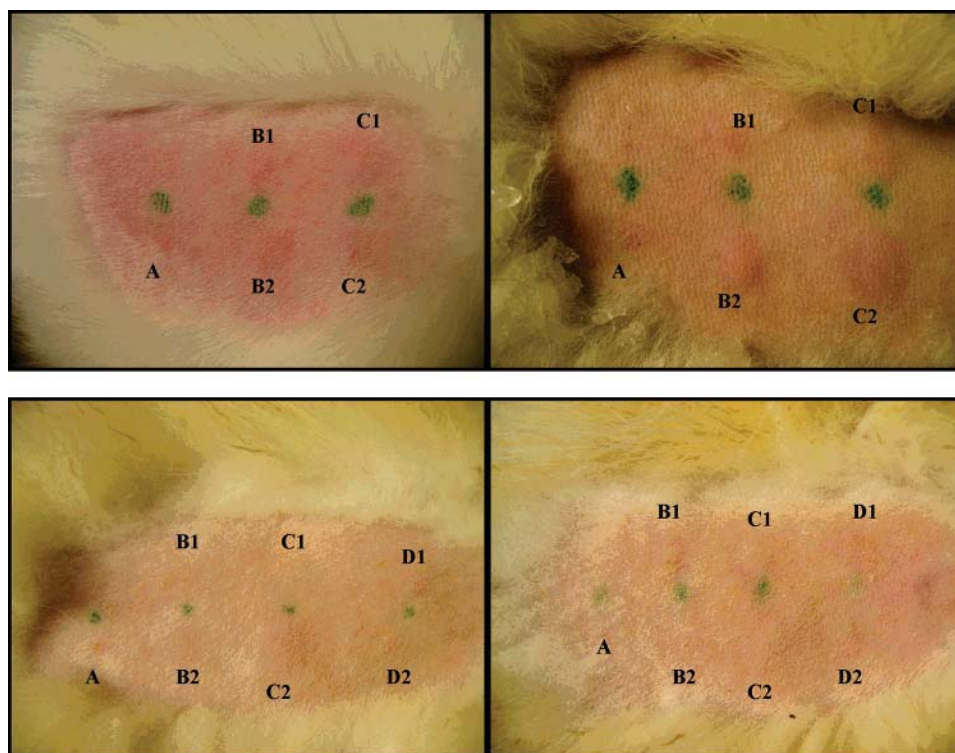
## Discussion

The delayed type hypersensitivity intradermal test results reflect the general ability of bitches and pups to induce CD4+ T lymphocyte proliferation (immune competence) and specific T cell response upon antigen stimulation (pups). The results from the present study showed that Con A was the most potent mitogen in the sledge dogs, and the stronger response in bitches compared to pups was probably due to maturation of the immune system (30). Another explanation for the stronger response in bitches could be the pups' shorter dietary exposure to polluted minke whale blubber.

The two groups were standardized regarding genetic variation (paired sisters), age, body condition, energy intake, and food composition (nutrition) which minimized such

impacts on the cellular dermal response (37). However, the concentrations of zinc, iron, selenium, and omega-3-fatty (O-3-FA) acids in minke whale blubber and pork fat differed (Table 2). Earlier investigations have stated that polyunsaturated fatty acids (n-3) have an unknown immuno modulating and suppressive effect (38), which is used prior to organ transplantations for minimizing the risk of tissue rejection (39). Similarly, studies of mercury (40) and OHCs (16, 17) have also shown immunosuppressive effects. We can therefore not distinguish among the immunosuppressive proportion from high levels of organochlorines and brominated flame retardants, low levels of mercury, and difference in fatty acid composition when evaluating whale blubber vs pork fat immunopathology. On the other hand, the present study is a real-life situation applying pollutants and polyunsaturated fatty acid environmental "cocktail" levels within a controlled study allowing extrapolation to wildlife and environmental medicine situations.

**Pathogenesis of OHC Immunosuppression.** The difference in lymphocyte proliferation capability after mitogen (PHA, Con A) stimulation between exposed and controls suggests a general potential immunosuppression effect from contaminants and fatty acids on lymphocyte proliferation (37, 38, 41). Additionally, the measurement of cell-mediated KLH immunity demonstrates a similar effect on cells and factors involved in a classical delayed type hypersensitivity T-lymphocyte response (37, 38, 41). No KLH dermal reactivity could be detected in exposed pups, indicating that dogs were either not sufficiently sensitized to KLH during the immunization or that the ability of the T lymphocytes to respond to KLH was suppressed at the time of performing the dermal test. The findings that both the response to T cell mitogens (Con A, PHA) and antigen (KLH) are suppressed shows that individuals exposed to blubber may have an impaired ability to raise a T-cell mediated immune response, due to chronic toxicity from OHC/mercury exposure and immunomodulating effects from n-3 fatty acids. Furthermore, the fact that not all differences were significant within the pup groups could be due to their shorter dietary exposure than that of the bitches.



**FIGURE 2.** Example of 24 h delayed hypersensitivity reaction (type IV) after intradermal test application of negative control, PHA and Con A in exposed bitch (upper left), control bitch (upper right), and negative control, PHA, Con A, and KLH in exposed puppy (lower left) and control puppy (lower right). The intradermal cellular reaction is clearly visible (left) increasing in the order negative control < PHA (50 ppm) < PHA (200 ppm) < Con A (50 ppm) < Con A (250 ppm) (right). See Figure 1 for application and abbreviations.

**OC Dose and Effect.** Several studies have investigated the negative impact from OCs on the immune system. Bilrha et al. (42) of piglets exposed to a PCB/OC pesticide seal blubber mixture showed an impaired in vivo antibody response to *Mycoplasma hyopneumoniae*, although an increased in vitro response to PHA was found, indicating immunopathy from PCB/OC pesticide exposure. Canadian studies on rats and mice fed PCB and OC pesticide contaminated beluga whale blubber from St. Lawrence River in Canada showed no immunotoxic effects (43), while a decrease in the CD8+ T cell population (spleen), reduced specific antibody response toward sheep red blood cells (SRBC), and reduced phagocytosis from peritoneal macrophages was found by Fournier et al. (44). It is possible that a similar CD8+ T cell as well as B cell immunotoxic pathogenesis is present in the West Greenland sledge dog but we have no samples to investigate such an impact. Furthermore, in the delayed hypersensitivity reaction the CD4+ T helper cells play a major role and these cells also are important for induction of antibody formation. The OC exposure may therefore also have an impact on the B-cell production of specific antibodies as well (32). The levels were environmentally relevant, though higher than those of the present dog study.

In studies of guinea pigs, mice, and monkeys, PCB exposure (industrial composition and dose higher than those of the present dog study) was shown to reduce the specific antibody response toward SRBC in vivo and vitro (45–47). PCB also reduced immune globulin levels in general (46) and the specific antibody response toward tetanus toxin (48). In a similar experimental study of rhesus monkey (*Macaca mulatta*), an inverse correlation was found between in vitro lymphocyte proliferation response (provoked by PHA and Con A) and low-dose PCBs (Aroclor 1254) of levels magnitudes higher than those used in the present study (49). Furthermore, a dose-related decrease in IgM and IgG response toward SRBC was observed and the overall pathogenesis was

hypothesized to be an alteration in T-cell and/or macrophage activity/function, as suggested for the present study. Finally, a human study of prenatal PCB and dioxin background levels magnitudes lower than those in the present study showed an increased susceptibility to infectious diseases, as well as antibody level changes, after the first vaccination (50). These controlled studies all support an immunotoxic effect from minke whale blubber pollutants in the West Greenland sledge dog.

Vos and Driel-Grootenhuys (51) and Vos and Van Genderen (52) measured the dermal reaction diameter after tuberculin injection in guinea pigs fed a PCB (Clophen A 60) dietary concentration of 50 ppm and found that the cell-mediated immunity was significantly reduced when compared to that of the control group. Similar effects from DDT and Aroclor 1254 were found in rabbits fed 150 and 170 ppm, respectively (53). Although significantly higher OC concentrations compared to the present study were used, it supports the immunotoxic effects in the present sledge dog study.

**Wildlife.** Few studies of wildlife mammals have suggested an immunotoxic effect from environmental OHCs. Lie et al. (15) suggested a relationship similar to in vitro induced lymphocyte proliferation, following stimulation with KLH, PHA, and Con A, in Svalbard polar bears relying on contaminated seal blubber (correlative studies). Samewise, Lie et al. (14) suggested that PCBs and OC pesticides may play a central immunosuppressive role in antibody response based on correlative studies of immunization of Svalbard and Canadian polar bears. The OHC levels ingested by the dogs in the present study are similar to those consumed by East Greenland and Svalbard polar bears (54). In striped dolphins and harbor seals, Troisi et al. (25) indicated that OHCs increased lung and uterus “Clara cell secretory protein”, which is involved in immunoregulatory and antiinflammatory in innate immunity, especially in lungs, uterus, and prostate. Similarly, a controlled study of harbor seals, one group fed with highly OHC-contaminated fish and one group fed



**TABLE 3. Results from the Intradermal Test of the 16 Bitches (P Generation) and Their 9 Pups (F1 Generation) in the Present Study, Divided by Type of Mitogen (PHA, 50/200  $\mu\text{g/mL}$ ; Con A, 50/250  $\mu\text{g/mL}$ ) and Antigen (KLH, 20/200  $\mu\text{g/mL}$ ) within Exposed and Control Group<sup>a</sup>**

Generation P (Bitches)				
dose ( $\mu\text{g/mL}$ )		exposed (mean $\pm$ SD) n = 8	difference	controls (mean $\pm$ SD) n = 8
<b>PHA</b>				
50	diameter	2.69 $\pm$ 2.63*	<	5.50 $\pm$ 2.20
	height	0.31 $\pm$ 0.37*	<	1.44 $\pm$ 1.43
	erythema	0.50 $\pm$ 0.38*	<	1.44 $\pm$ 1.02
200	diameter	3.63 $\pm$ 2.39	<	6.25 $\pm$ 2.44
	height	0.69 $\pm$ 0.53	<	1.38 $\pm$ 1.71
	erythema	0.75 $\pm$ 0.54	<	1.56 $\pm$ 1.32
<b>Con A</b>				
50	diameter	6.00 $\pm$ 2.20	<	8.00 $\pm$ 2.62
	height	1.13 $\pm$ 0.69	<	2.00 $\pm$ 1.49
	erythema	1.13 $\pm$ 0.64	<	1.94 $\pm$ 1.18
250	diameter	6.38 $\pm$ 2.39*	<	10.4 $\pm$ 2.72
	height	1.88 $\pm$ 0.79	<	2.81 $\pm$ 1.62
	erythema	1.81 $\pm$ 0.70*	<	2.75 $\pm$ 1.39
<b>Generation F1 (Pups)</b>				
dose ( $\mu\text{g/mL}$ )		exposed (mean $\pm$ SD) n = 5	difference	controls (mean $\pm$ SD) n = 4
<b>KLH</b>				
20	diameter	0.00*	<	2.63 $\pm$ 1.89
	height	0.00*	<	0.88 $\pm$ 0.63
	erythema	0.00*	<	0.88 $\pm$ 0.25
200	diameter	0.00*	<	4.00 $\pm$ 3.32
	height	0.00*	<	0.80 $\pm$ 0.45
	erythema	0.00*	<	1.10 $\pm$ 0.82
<b>PHA</b>				
50	diameter	1.13 $\pm$ 1.33*	<	2.1 $\pm$ 1.03
	height	0.38 $\pm$ 0.48	<	0.9 $\pm$ 0.42
	erythema	0.25 $\pm$ 0.29	<	0.7 $\pm$ 0.45
200	diameter	4.25 $\pm$ 3.12	>	2.7 $\pm$ 1.30
	height	1.00 $\pm$ 0.71	<	1.1 $\pm$ 0.82
	erythema	1.00 $\pm$ 0.82	>	0.7 $\pm$ 0.45
<b>Con A</b>				
50	diameter	4.63 $\pm$ 1.11	<	5.5 $\pm$ 5.03
	height	1.13 $\pm$ 0.75	<	1.9 $\pm$ 1.24
	erythema	1.00 $\pm$ 0.71	<	1.5 $\pm$ 0.87
250	diameter	7.13 $\pm$ 1.93	>	6.9 $\pm$ 1.88
	height	1.75 $\pm$ 0.29	<	2.5 $\pm$ 1.06
	erythema	1.88 $\pm$ 0.63	>	1.6 $\pm$ 0.82

<sup>a</sup> Reactions are given in mean diameter (mm), mean height score (0 to +4), and mean erythema score (0 to +3) for 24 h reactions (48 h reactions were excluded as all were magnitudes lower compared to 24 h). \* = Significantly lower intradermal reaction in the exposed group compared to the corresponding control at  $p \leq 0.05$ .

relatively uncontaminated fish, showed that the exposed group had significantly lower in vitro innate (NK cell) proliferation against PHA and Con A and lower acquired in vivo (T cell) proliferation toward specific antigens (tetanus toxin) (18–22). However, the results could not be reproduced in a controlled study on rats fed a diet containing 33% Baltic Sea herring (23), and the authors concluded that the harbor seal might be specifically sensitive to OHC immunotoxicity (21). In the St. Lawrence beluga whale, pathology (neoplasms, pneumonia, opportunistic infections, and mastitis) and in vitro reduced proliferation of beluga whale lymphoid cells exposed to mixtures of OHCs at environmentally relevant concentrations have suggested a relationship between OHCs and immunosuppression (26–28). In the study by Lahvis et al. (24) on free-ranging bottlenose dolphins, an inverse correlation between an in vitro lymphocyte response to

mitogens (PHA/Con A) lymphocyte proliferation and OHCs was found, however, the results were based on 5 individuals. In addition, the OHC concentrations were measured in blood, which may not reflect the general exposure, but recent OHC intake (55) or mobilization of periphery fatty energy resources during fasting (31). These studies all support our findings in the present West Greenland sledge dog study.

**Potential Impact and Future Considerations.** Why are the effects from polluted marine mammal blubber rich in n-3 fatty acids on the immune system of wildlife and humans interesting at all? An impairment of this part of the immune system by minke whale blubber may lead to decreased resistance to infections caused by microbial pathogens (47, 56–58). On the basis of the results of the present dog study, and the scientific literature, Arctic wildlife species and Inuits may suffer from cellular immunosuppressive environmental OHC exposure.

The immuno depressing effect from eating minke whale blubber (rich in contaminants and n-3 fatty acids) on the specific and nonspecific cellular immunity, raising concern for decreased resistance to diseases, is new and important information. This knowledge should be used in future environmental medicine studies of xenoendocrine disruptors in humans and in polar bear conservation strategies as chemical and climate changes are predicted to change dramatically in the coming years, adding other immunosuppressive starvation stressors (59, 60). As food resources are depleted by thinning sea ice due to climate change, more research should assess the forecasted rise in additive immunopathy effects in polar bears. Additionally, our study demonstrates the importance of analyzing the combined effects of anthropogenic pollutants and fatty acid composition when investigating immunotoxicity in future environmental studies as it may have importance for the most susceptible individuals.

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The potential use of lipids and stable isotopes as a source of information related to the West Greenland marine ecosystem (62°N to 72°N) including man, was investigated. Analysis were performed on marine tissues representing invertebrates, fish, seabirds and marine mammals as well as traditional meals from a local community. One part of the study also included minke whale samples from other part of the North Atlantic. Our results suggest a great potential in lipids and stable isotopes as a source of information in research issues related to a sustainable exploitation and management of the West Greenland marine ecosystem and to public health issues in Greenland. The results fill out an existing gap in our knowledge about the marine food web structure and trophic relations and add a potential new tool to improved management of large whales. In addition, data will be important when giving dietary recommendations, balancing the risk from the contaminants and the healthpromoting fatty acids in the traditional diet of Greenlanders.