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Body composition, field metabolic rate and feeding ecology of walrus (*Odobenus rosmarus*) in northeast Greenland

PhD thesis

Mario Acquarone

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Abstract: The Field Metabolic Rate (FMR) of two free-living, adult, male Atlantic walruses (*Odobenus rosmarus*) was measured by the Doubly Labelled Water isotopic technique in NE Greenland. Estimates of FMR were 345 MJ day^{-1} for a 1,370 kg and 417 MJ day^{-1} for a 1,250 kg walrus (mean = 381 MJ day-1) reflecting a difference in diving activity measured by ARGOS© Satellite-Linked Transmitters and Time-Depth Recorders. Converted to walrus food equivalents these quantities correspond to 68 and 82 kg day^{-1} mussel shell-free fresh matter, respectively, representing 5.0 and 6.6 % day^{-1} of their total body mass (TBM). These values agree with the estimates of bivalve mollusc intake derived from underwater, visual observations and activity data obtained by ARGOS© Satellite-Linked Dive Recorder of one walrus feeding in the wild (57 kg day^{-1} shell-free fresh matter, 4.7 % day^{-1} for TBM of 1226 kg). From this information and from data on local prey abundance, walrus predation in the Young Sound study area in NE Greenland appears to be lower than the carrying capacity. Deuterium Oxide dilution was employed on seven animals to estimate Total Body Water (TBW) content (56.8 % of TBM) and Water Turnover (44.5 g kg^{-1}day^{-1}) to obtain essential data for the calculation of the minimum effective dose of Doubly Labelled Water. TBW measures thus obtained and general allometric equations of body composition estimated body fat to 24.7 % of TBM which did not differ significantly from the estimates of blubber content obtained from the dissection of walruses in the wild. Chemical immobilization by etorphine HCl reversed by diprenorphine HCl has been employed to restrain the animals for instrument attachment. During isotope enrichment, the immobilization time has been prolonged to up to 6.75 hr. by use of medetomidine HCl.

Keywords: walrus, *Odobenus rosmarus*, Northeast Greenland, body composition, isotope dilution, field metabolic rate, doubly labelled water, feeding ecology, chemical immobilization, etorphine, diprenorphine, medetomidine.

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Contents

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Copenhagen, November 2003

Mario Acquarone

Summary

The quantification of food requirements is an important key for understanding the role of animals within their ecosystem, but it represents also essential information necessary to evaluate the responses of these organisms to changes in their natural environment and hence fluctuations in food availability.

It is beyond doubt that the climatic conditions in the North Atlantic have been variable throughout history and that the arctic regions in this area show local amplifications of climatic changes. The arctic ecosystems are simple compared to their temperate, subtropical and tropical counterparts. This makes them particularly vulnerable in case of rapid changes. For this reason the effects of any variation in the global climatic conditions are necessarily amplified in the Arctic.

The east coast of Greenland represents an interesting climatic gradient because of its north-south orientation. The ecosystems range from polar, to high arctic and to low arctic. It is in the inshore areas and the deep fjords that a part of the small east Greenland walrus population comes to feed and moult every summer. The sea surface above the rich bivalve mussel beds is covered with a thick layer of ice during most of the year, but during summer, for a short window of about three months, the ice melts away and allows access to this important walrus food resource. Therefore any change in the timing of the break-up and formation of the sea-ice has a profound impact on food availability for these animals.

It has long been known that walruses feed on the main bivalve species (e.g. *Mya* sp., *Hiatella* sp. and *Serripes* sp.) of these fjord's benthic communities. Analysis of the stomach content of the animals killed in occasion of the traditional Inuit hunt or by European sealers has provided strong evidence in this direction. However, recent studies have demonstrated that stomach contents only provide a partial picture of the feeding habits of these animals. Even for a walrus that has just been feeding, the action of the gastric secretions is extremely rapid and some benthic organisms, as polichetes and holoturians, are almost completely dissolved before dissection can begin, while the most resistant, as bivalve mollusks, are at best partially digested. For a complete quantification of the walrus diet it is therefore necessary to adopt other approaches.

The main focus of this thesis is the quantification of food requirements in free-living walruses. Two different methods have been adopted to attain this goal: (a) the direct underwater observations of walrus feeding on the mussel beds and (b) the estimate of energy consumption by the Doubly Labeled Water isotopic technique.

In case of the first approach, the observations of foraging walrus made by brave divers have allowed the retrieval of empty bivalve shells after feeding dive. By coupling this information with data on the frequency of the feeding dives obtained both by direct

observations and by satellite telemetry has resulted in the estimate of the average daily bivalve food consumption (shell free, wet weight) at 4.7% of the animal's total body mass.

The second approach involved the calculation of the animal's Field Metabolic Rate from measures of the washout rate of two stable isotopes tracers (^2H and ^{18}O) that had been injected in the animals at the beginning of the study. From these estimates of the average energy utilized over a period ranging from five to nine days, and from the energy content of the bivalve diet it has been possible to calculate a corresponding bivalve food consumption of 6-7% (shell free, wet weight) of the animal's total body mass.

These data have been utilized for the calculation of the impact of walruses on the benthic bivalve community in the Young Sound area. The interest in this area is due to the intense climate-related research and monitoring effort both by Danish and international scientists in relation to the Zackenberg environmental baseline monitoring station. From the results of this study walrus predation amounts to 1.2-1.3% of the standing biomass in the estimated walrus feeding area of their two most important food items (*Mya* sp. and *Hiatella* sp.). These values correspond to 19-28% of the annual production of these bivalves. Therefore, a climatic change causing an extension of the open water season, presumably would not immediately bring this ecosystem to the limit of its carrying capacity.

In the course of this work it has been necessary to repeatedly immobilize the study animals and two papers fully account for these procedures. One paper describes for the first time multiple immobilizations of the same individual and the other presents a novel technique especially useful for prolonged restraint for up to 6.5 hr. Furthermore, in order to prepare the Doubly Labeled Water study and minimize its costs, it was decided to perform a study of the body water metabolism by isotope dilution. The paper derived from this study presents novel data on body water pool size and body water turnover rate, which were used to estimate body composition which does not present significant differences with data obtained from dissection (in other studies).

Dansk resumé

Bestemmelse af dyrs fødebehov er vigtig for at kunne forstå deres rolle i økosystemet. Desuden er denne information essentiel for at kunne vurdere, hvordan dyr reagerer på ændringer i deres omgivelser og ændringer i fødegrundlag.

Der er ingen tvivl om, at klimaet i Nordatlanten har varieret betydeligt igennem tiden, og at de arktiske områder har haft lokale klimasvingninger. Det arktiske økosystem er simpelt og dermed også mere sårbart sammenlignet med tempererede, tropiske og subtropiske områder. Af denne grund vil enhver ændring i det globale klima nødvendigvis blive forstærket i Arktis.

Grønlands østkyst repræsenterer på grund at sin nord/sydgående orientering en interessant klimatisk gradient. Økosystemet strækker sig fra Polarhavet over højarktis til lavarktis ved Kap Farvel i syd. Det er langs disse kystnære områder med dybe fjorde en del af den lille østgrønlandske hvalrosbestand søger føde og fælder hver sommer. Det meste af året er havet over muslingebankerne dækket af et tykt lag is. I ca. tre måneder hver sommer smelter isen og giver hvalrosserne mulighed for at udnytte den vigtige føderessource, som muslingebankerne udgør. Derfor vil ændringer i isens smeltetidspunkt have stor betydning for hvalrossernes adgang til føde.

Det har længe været kendt, at hvalrosser lever af de vigtigste muslingearter (bl.a. *Mya* sp., *Hiatella* sp. og *Serripes* sp.) i disse fjordes bentiske samfund. Dette understøttes af analyser af maveindhold fra hvalrosser, der er skudt af lokale grønlændere eller af europæiske sæljægere. Nye studier har dog vist, at maveundersøgelser kun fortæller en del af historien om hvalrossernes spisevaner. Selv hos en hvalros der lige har spist er mavesekretionen så kraftig og nedbrydningen så hurtig, at nogle bentiske organismer som børsteorme og søpølser næsten er forsvundet, før dyret er skåret op, mens muslingerne, som er svært nedbrydelige, kun er delvist nedbrudt. For at få et komplet billede af hvalrossernes føde er det nødvendigt at anvende andre metoder.

Hovedformålet med denne afhandling er at kvantificere fødebehovet for fritlevende hvalrosser. To forskellige metoder har været anvendt: (a) undervandsobservationer af hvalrosser der fouragerer på muslingebanker og (b) energiforbruget målt ved hjælp af dobbeltmærketvand isotopteknik.

Under den første metode blev fouragerende hvalrosser observeret af en dykker, som bagefter indsamlede de tomme muslingeskaller. Ved at estimere muslingernes oprindelige vægt og koble dette med antallet af fødesøgningsdyk fra direkte observationer og fra data fra satellitsendere, var det muligt at estimere det gennemsnitlige daglige fødeindtag af muslinger (vådvægt uden skal) til 4,7% af dyrets vægt.

I den anden metode blev dyrets energistofskifte målt ved at injicere to stabile isotoper (2H og 18O) og måle deres udvaskningsrate fra kroppen. Ved at beregne det gennemsnitlige energiforbrug over fem til ni dage og sammenholde det med energiindholdet i muslingerne, er det muligt at beregne fødeindtaget af muslinger til 6-7% (vådvægt uden skal) af hvalrossernes kropsvægt.

Disse data er brugt til at beregne hvalrossernes påvirkning af det bentiske muslingesamfund i Young Sund området. Interessen i dette område er særlig stor, fordi der foregår en lang række danske og internationale klimarelaterede forsknings- og overvågningsprojekter. Disse projekter udføres i forbindelse med Zackenberg forskningsprogram. Fra resultaterne opnået i nærværende studie har det vist sig, at hvalrosserne fortærer 1,2-1,3% af biomassen af de to vigtigste fødeemner (*Mya* sp. and *Hiatella* sp.) i deres fourageringsområde. Dette svarer til 19-28% af den årlige biomassetilvækst for de to muslingearter. Derfor vil stigende temperaturer med følgende længere åbenvandsperiode ikke umiddelbart betyde, at økosystemet når grænsen for, hvad det kan bære med hensyn til hvalrosser.

I løbet at dette studie har det været nødvendigt at bedøve hvalrosserne gentagne gange, hvilket er beskrevet grundigt i to af de vedlagte artikler. Den ene artikel beskriver gentagne bedøvelser af de samme hvalrosser, mens den anden beskriver en ny teknik udviklet til at holde dyrene bedøvede i længere tid (op til 6,5 time). Endelig var det nødvendigt at gennemføre et studie med enkeltmærket vand for at beregne dyrets vandomsætning. Derved kunne den mindst mulige mængde af det kostbare dobbeltmærkede vand, der var nødvendig for at gennemføre dette studie, efterfølgende beregnes. Artiklen over vandomsætningen præsenterer nye data over hvalrossers vandmængde og omsætning, som blev brugt til at bestemme kropssammensætningen. Kropssammensætningen bestemt ved enkeltmærket vand og ved dissektion (udført i et andet studie) viste sig at være sammenlignelige.

(*Oversættelse til dansk af Jonas Teilmann og Kirsten Rydahl, DMU*)

Kalaallisut eqikkaaneq

Uumasut pinngortitami inuunerat paasisinnaassagaanni uumasut nerisassatigut pisariaqartitsinerannik missingersuisinnaaneq pingartuuvoq. Paasissutissarlu tamanna uumasut avatangiisaat nerisassaallu allanngoraanngata qanoq qisuariartarnerinut nalilersuisarnermut aamma pingaaruteqarpoq.

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Paasissutissat tamakku Young Sund-ip eqqaani aarrit uiloqarfinnik sunniisarnerannut naatsorsuinermut atorneqarput. Tamatuminnga soqutiginninneq annertoorujussuuvoq, tassami tamaani qallunaanit nunanillu allanineersunit silasiornermut atatillugu ilisimatusarnerit misissuinerillu ingerlanneqarmata. Suliat tamakku Zackenbergimi ilisimatusarnernut assigiinngitsunut atatillugu ingerlanneqarput. Misissuinermi matumanerpiaq inernerit tunngavigalugit paasineqarsimavoq, uillut pingaarnerit marluk (*Mya* sp. and *Hiatella* sp.) 1.2-1.3%-ii aarrit nerisassarsiortarfimminni nerisaraat. Tassa uillut taakku marluk ukiumut amerleriaatigisartagaasa 19-28%-eraat. Taamaattumik silaa kianneruleriartortillugu sivisunerusumik imaasalernera aarrit eqqarsaatigalugit pinngortitap nerisassaqassutsimik nammassisinnaasaata killissaata angunissaanut maannangaaq sunniuteqarnavianngilaq.

Misissuinerup matuma ingerlanerani aarrit arlaleriarlugit ilisimajunnarsinneqartariaqartarsimapput, tamannalu allaatigisani marlunni ilanngunneqarsimasuni sukumiisumik allaaserineqarsimavoq. Allaatigisami siullermi aarrit assigiit arlaleriarlugit ilisimajunnaarsinneqartarnerat allaaserineqarpoq tullianilu uumasunik sivisunerusumik ilisimajunnaarsitsisarnermi (akunnerit 6,5-mit tikillugit) periaaseq nutaaq allaaserineqarpoq. Kiisalu uumasut qanoq imertortiginerat naatsorsorniarlugu misissuineq imeq isotopimik ataatsimik nalunaaqutserneqarsimasoq atorlugu ingerlanneqartariaqarsimavoq. Taamaalilluni imeq isotopinik marlunnik nalunaaqutserneqarsimasoq akisoqisoq pisariaqartoq minnerpaaq misissuineq naammassiniarlugu kingorna naatsorsorneqarsinnaasimavoq. Allaatigisaq qanoq imertortiginerannik allaatiginnittoq aarrit imeqassusaannik imermillu atuinerannik paasissutissanik nutaanik saqqummiivoq, taakkulu timaasa katitigaanerannik aalajangiiniarnermi atorneqarsimavoq. Timaasa katitigaanerisa naatsorsorneqarnera imeq isotopimik ataatsimik nalunaaqutserneqarsimasoq atorlugu pilattarlugillu misissoqqissaarlugit (misissuinermi allami ingerlanneqartoq) ingerlanneqarnera imminnut naleqqiunneqarsinnaasimapput.

Structure of this work

This thesis is composed of an introductory section and six papers dealing both with the necessary task of transforming free-living walruses into manageable and docile creatures through chemical restraint (2 papers), with the essential physiological parameters of water metabolism together with the calculations that can be derived from water isotope dilution (2 papers) and finally with the ecological aspects that can be inferred through behavioural observations and integration of the results obtained with studies of other ecosystem components (2 papers).

List of publications included in this thesis

Paper I. **Acquarone M, Born EW, Griffiths D, Knutsen LØ, Wiig Ø, Gjertz I. Evaluation of etorphine HCl reversed by diprenorphine HCl for immobilization of free-ranging Atlantic walrus (*Odobenus rosmarus rosmarus* L.*)*.**
Describes and evaluates the use of etorphine, with its antagonist diprenorphine, as a primary immobilizing agent for free-living Atlantic walruses. This work includes the most complete set of data to date on this technique and provides an evaluation of the efficacy of this narcotic for restraining walruses and allowing approach and manipulation.

Paper II. **Griffiths D, Born EW, Acquarone M. Prolonged chemical restraint of walrus (*Odobenus rosmarus*) with etorphine/ diprenorphine supplemented by medetomidine/ atipamezole.**
Describes the use of medetomidine as a supplement to etorphine in cases where prolonged restraint of walruses is needed. Medetomidine has proven essential for the restraint of walruses for repeated blood sampling and physiological monitoring.

Paper III. **Acquarone M, Born EW. Body water and body composition of free-ranging Atlantic walruses (*Odobenus rosmarus rosmarus* L.*)* studied by isotope dilution.**
Novel data on the water metabolism of walruses inferred through water isotope dilution are presented here. This study has provided basic water metabolism parameters and essential background data for the Doubly Labeled Water (DLW) study (paper IV).

Paper IV. **Acquarone M, Born EW, Speakman JR. Direct measures of pinniped field metabolic rate: implications for fisheries models.**
Reports the measure of energy metabolism of two walruses through the DLW technique. These measures can be utilized to infer average energy needs and food intake for free living animals. It is the first time that this technique is employed successfully on animals larger than 114 kg.

Paper V. **Born EW, Rysgaard S, Ehlmé G, Sejr MK, Acquarone M, Levermann N. Underwater observations of foraging free-living Atlantic walruses (*Odobenus rosmarus rosmarus*) and estimates of their food consumption. Polar Biol 2003; 26:348-357.**
Infers walrus food intake from direct underwater observations of feeding animals. From the examination of the remainders of feeding dives it has been possible to define the average meal size and meal composition with respect to bivalve mollusks.

Paper VI. **Born EW, Acquarone M. An estimation of walrus (*Odobenus rosmarus*) predation on bivalves in the Young Sound area (NE Greenland). Meddr Grønland, Biosci. Accepted for publication.**
Presents an integrated study coupling information on the benthic communities and novel data on walrus feeding from the other studies in this work to assess the impact of walrus feeding on the Young Sound bivalve community.

Introduction

Recent studies have observed a global temperature increase since the 1970's (Moritz et al. 2002). In the Arctic, a corresponding reduction of the extent and thickness of sea ice has also been observed (Rothrock et al. 1999; Johannessen et al. 1999; Kerr 2002). Especially ice extent, calculated from sea ice concentration monitored continuously from satellite microwave sensor measurements, has diminished since 1978. The data show a decrease in the Northern Hemisphere annual sea ice area by 7.4% between 1978 and 2002. The decrease is larger in summer: 14% in September versus 5% in March (Johannessen et al. 2002) leading to a reduction in the multi-year ice by 7-9% over the past two decades (Johannessen et al. 1999; Comiso 2002). According to some climate models (ECHAM4 and HadCM3) this trend will not only continue, but also accelerate in the Arctic compared to temperate and tropical regions (Moritz et al. 2002).

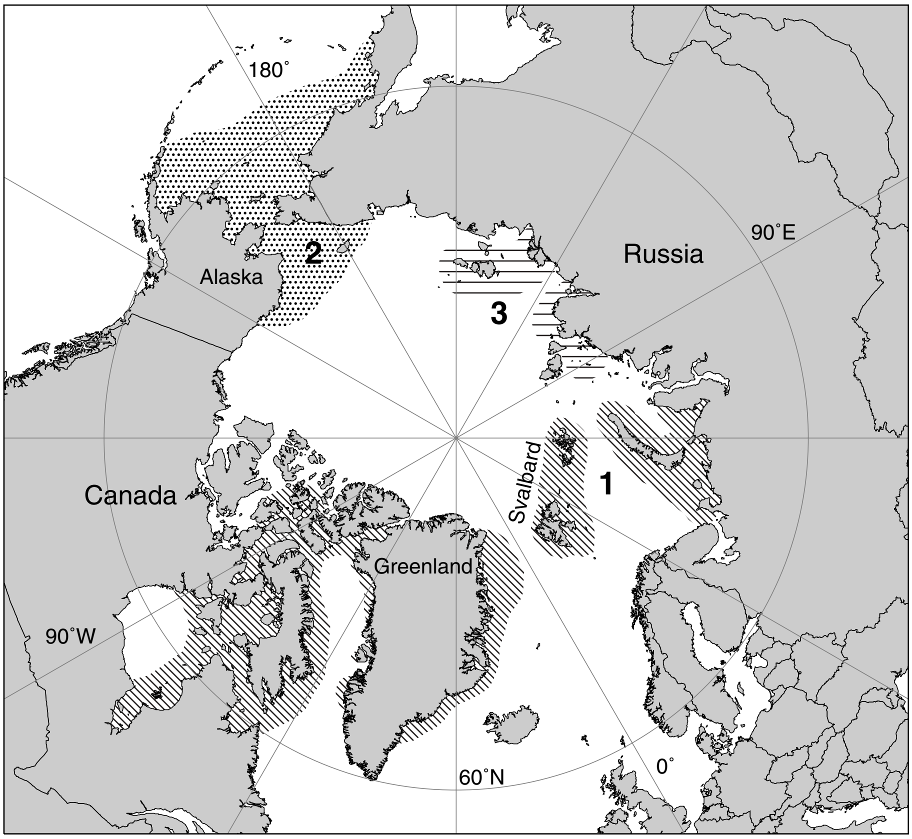


Figure 1 Approximate present distribution of walrus subspecies: Atlantic walrus (1), Pacific walrus (2) and (uncertain status) Laptev walrus (3) (modified from Fay 1985; in Born et al. 1995).

Both these models forecast a lengthening of the open-water season, but irrespective of the direction of climate change, a variation in the extent and the timing of break-up and formation of the yearly ice cover is expected to have a dramatic effect on the arctic marine ecosystem. Hence the need for detailed ecosystem modeling for this area.

One of the mammalian predators in the Arctic is the walrus (*Odobenus rosmarus*), a Holarctic pinniped species. The southern limits to its distribution are represented by the pack ice. Presently it regularly occurs as far south as the Aleutian Archipelago in the

Bering Sea, the northern coast of Labrador, Central Greenland, Svalbard in the Atlantic and the Siberian coastline in Northern Asia, although occasionally individual walruses have been observed in more temperate regions as along the shores of northern Europe. Walruses occur in two subspecies (Figure 1). The Pacific walrus *O.r.divergens* (Illiger, 1815) in the north Pacific, Bering Strait, Beaufort sea and the Atlantic walrus *O.r.rosmarus* (Linnaeus, 1758), in the north Atlantic from the Canadian Arctic to the Svalbard archipelago which comprises different subpopulations. A third subspecies is suggested by some authors and questioned by others (for an overview see: Born et al. 1995): the Laptev Sea walrus *O.r.laptevi* Chapskii, 1940 which resides between the Kara Sea, the East Siberian Sea, the northern Siberian coast and the pack ice. Information from studies of distribution and movement (Born and Knutsen 1997) and from genetic analysis (Andersen et al. 1998; Born et al. 2001) show that the East Greenland Atlantic walrus subpopulation is mostly isolated from the neighboring populations and numbers around 1000 individuals (Born et al. 1997) which makes it particularly sensitive to any disturbance (of climatic or of human origin).

Walruses were presumably abundant in eastern Greenland and were subjected only to limited traditional subsistence hunt by the local Inuit population until the European commercial hunt begun in 1889 (Born et al. 1997). The hunt records show that 1680 walruses were killed by European sealers between 1889 and 1955 (Born et al. 1997). As a consequence the population was heavily reduced. Protection was introduced in 1956. Apparently the population has grown slowly since and the most recent size estimate of around 1000 individuals (Born et al. 1997) is probably still valid. The reasons for the slow recovery are probably natural biological factors, such as limited access to the inshore feeding areas (Fay 1982; Born et al. 1997). Although the hunters of Ittoqqottormiit and Ammassalik continue a minimal, local exploitation of walruses, this hunt is presumably sustainable (Glahder 1995; Born et al. 1997).

During summer walruses feed intensively (Fay 1982; Born et al. 1997) presumably to sustain body growth, maintain body condition, match the metabolic cost of molting and for heat production but also for accumulating blubber as energy storage, before the winter months when they are precluded access to the inshore feeding grounds and food availability is lower. In the summer period they rely on access to stable haul-out platforms in the vicinity of the feeding grounds to minimize the cost of transport and thus maximize feeding efficiency (Born et al. 1995). These platforms are preferably residual ice floes floating in the vicinity of the mussel banks, but when these disappear, walruses must revert to terrestrial haulouts.

Walruses exhibit a high degree of site fidelity to the terrestrial haulouts to the point of returning to the same beach year after year (Miller 1976; Salter 1979; Fay 1982; Born and Knutsen 1992b; Born and Knutsen 1997) and they appear to be particularly dependent on access to "traditional" feeding grounds in the vicinity of these haulouts. In Greenland there are only two active regular such haulout sites: one at Sandøen (74°15.7′N 20°09.7′W) and one at Lille Snenæs (76°52.7′N 19°37.9′W) (Figure 2). The present work was carried out at both sites. This behavioral trait provides an ideal background for studies involving multiple recaptures of the same individual. In previous work several individual walruses have been identified and resighted in the following years at the Lille Snenæs haulout (Born et al. 1997).

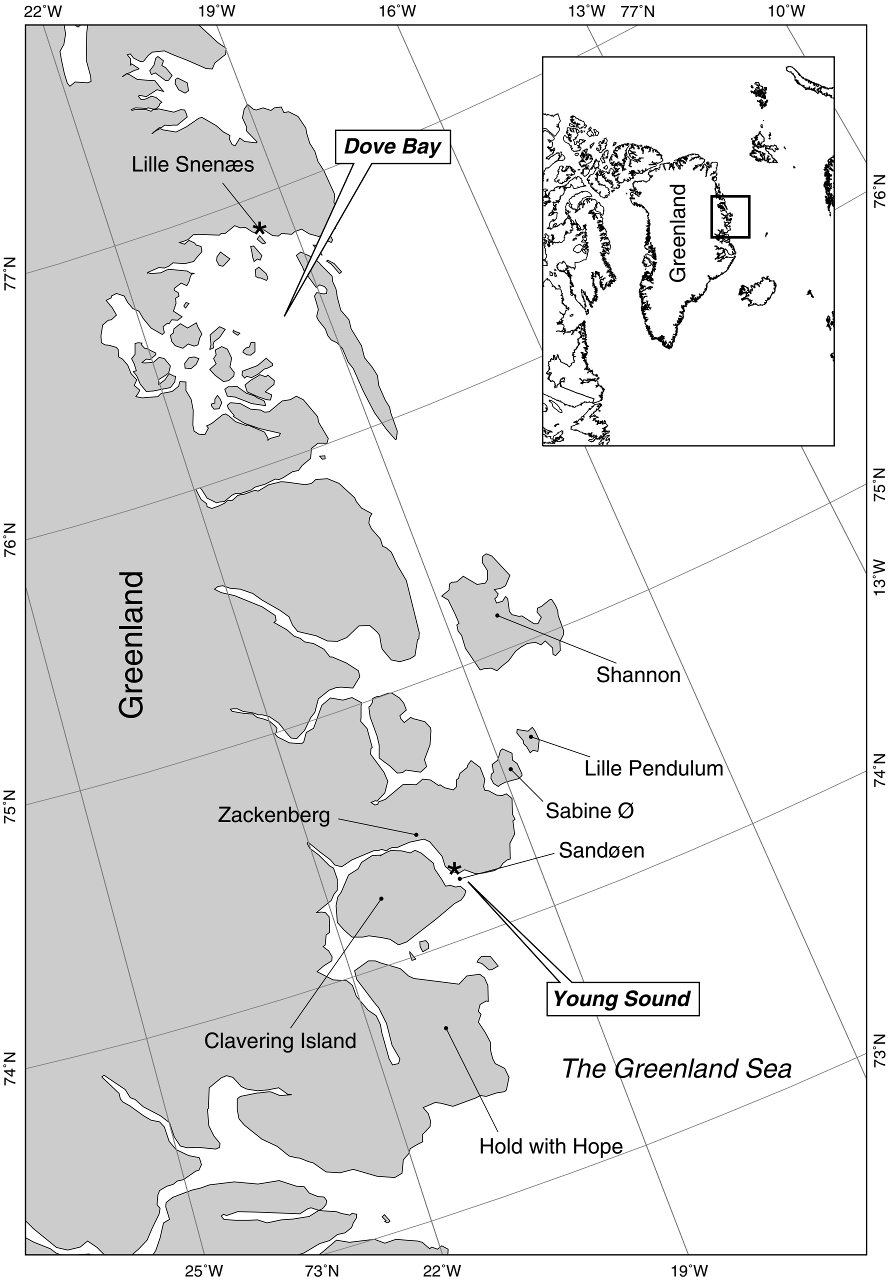
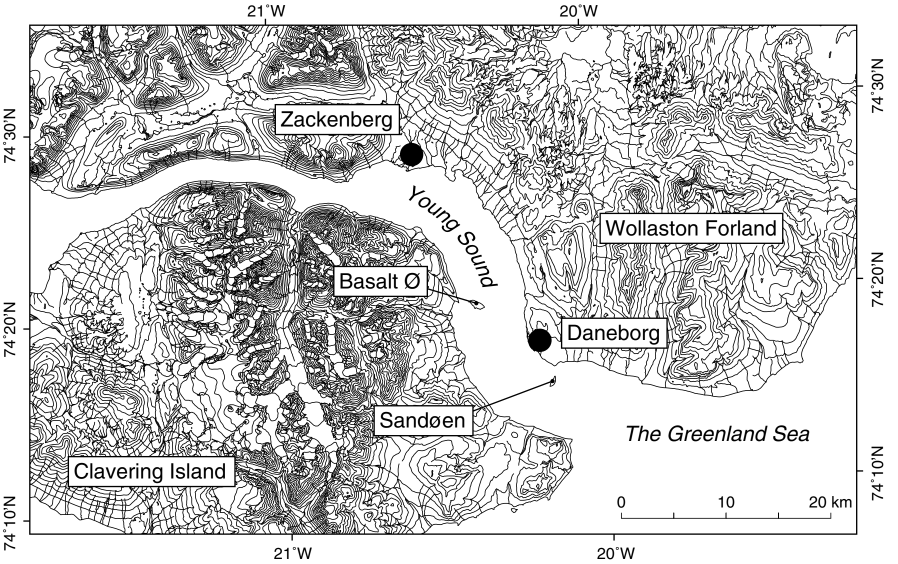


Figure 2 Map of the study area in NE Greenland. The Sandøen and Lille Snenæs haulouts are indicated (*).

Walrus distribution and abundance could be connected to, and limited by climate through thermoregulatory behavior and physiological characteristics (Fay and Ray 1968; Ray and Fay 1968). However the importance of the spatio-temporal availability of walrus food resources, and thus the range and the rate of increase of the walrus population, should not be underestimated. Generally speaking, for the inshore areas comprising the walrus feeding grounds, a warmer climate could result in a longer open-water period and increased accessibility to food resources. A colder climate could have the opposite effect and shorten the effective feeding season. Hence, there is a need to quantitatively estimate food requirements for this species, which is one of the goals of this thesis.

Figure 3 Map of Young Sound. The Sandøen haulout is visible at the eastern end of the fjord close by the military base Daneborg. Basalt Ø is the only major island inshore in the fjord. On the northern shore the Zackenberg research station.



In 1995 a project called Changes in Arctic Marine Production (CAMP) was initiated, coordinated by the Danish National Environmental Institute, to investigate marine production and the related carbon cycle in Young Sound, a high Arctic Fjord ecosystem (Figure 3), (http://www.dmu.dk/lakeandestuarineecology/camp/) with special attention to those elements of the ecosystem most sensitive to climate-induced changes. The CAMP project focused on the quantification of carbon flow through the major components of the Young Sound ecosystem in order to provide baseline measurements to understand the ecosystem's sensitivity to climatic changes. Great effort has been made to in order to obtain data from the ecosystem's most conspicuous components (e.g. in Rysgaard et al. 1996; Rysgaard et al. 2003b) among which the walruses. For walruses, it was deemed essential to accurately quantify feeding requirements as input data for a model of energy and material balance in the fjord ecosystem. Hence the broader context of this thesis.

Most of the information on walrus feeding in the wild comes from the analysis of stomach contents. For Pacific walruses the information has been summarized by Fay (1982), while for Atlantic walruses generic indications from stomach content analysis on the diet quality and quantity can be found in Vibe (1950), Mansfield (1958), Gjertz and Wiig (1992) and Fisher and Stewart (1997). The principal food items of walruses are benthic bivalve mollusks (Fay 1982; Fay and Burns 1988). Seals (Fay 1982; Lowry and Fay 1984), fish (Born et al. 1995; Born et al. 1997) and seabirds (Gjertz 1990) occur occasionally in the walrus diet (Figure 4). However, there are strong indications that analysis of the stomach content of walruses might not represent a complete picture of the ingested food items. An in vitro digestion study simulating the effect of walrus gastric fluids on a sample of the benthic community from a walrus feeding ground has shown that a stomach sample may neither reflect the species composition and size of the prey consumed nor is volume a reliable measure of a preys relative importance in the meal (Sheffield et al. 2001). In conclusion stomach content analysis does not always allow a reliable quantification of food consumption. Walrus feeding though has been investigated in detail for animals in captivity. Using data gathered for husbandry purposes Kastelein et al. (2000) have described the artificial diet fed to the walruses at the Harderwijk Marine Mammal Park in Holland. Although this study was not based on a natural walrus diet in a natural habitat, it is the most detailed account of walrus food requirements to date. It includes record of the animals' food intake (composition and quantity), physiological condition of the animal (age, dimensions) and of the physical settings (weather and pool characteristics) during the whole measurement period which ranged from 1 to 14 years. Therefore, this thesis supplies previous knowledge of walrus feeding with robust novel indications of food requirements for free-ranging animals.

The isotope dilution studies have been carried out at the Lille Snenæs haulout, which is located some 270 km north of Young Sound along the East Greenland coast (Figure 2). These studies had been already attempted in August 1999 on Sandøen, in Young Sound, with no success due to the very low number of walruses frequenting the haulout. Probably the unusual amount of sea-ice that drifted in the fjord from offshore that year, and often blocked access to the island, offered a more congenial haulout platform than the beach. Therefore, to ensure the success of the project in spite of the additional costs, it was decided to relocate the study at the more stable Lille Snenæs haulout in northern Dove Bay (Born and Knutsen 1992b; Born and Knutsen 1997). The behavior (activity levels, trip length and duration) and energy requirements of the walruses studied at Lille Snenæs have no reason to be different from walruses from Sandøen. There are strong indications that the groups at Sandøen and at Lille Snenæs experience some degree of contact. In this and previous studies individual animals have been identified at both haulouts during the same field season (e.g. in Born et al. 1997). It is therefore assumed that the measures of metabolism from the northern location can be directly applied to the population utilizing Young Sound and elsewhere in the Arctic.

In this thesis it was decided to take two different approaches to estimate food requirements for free-ranging walruses. One focused on measuring the typical meal size. It involved diving to a walrus feeding-patch to record the extent of predation (Paper V). By coupling this measure to the analysis of behavior recorded by satellite telemetry, which provided data on the frequency of the feeding dives, it was possible to estimate of food consumption. The other approach involved the indirect calculation of food requirements from measurements of the energy demands of the individual animals performed by the Doubly Labeled Water (DLW), isotope dilution technique (Paper IV). This second approach provided an estimate of the energy expended by the animal integrated over a period of time of up to nine days. Assuming that feeding is not restricted, in other words the animal feeds at will, the energy expended during the measurement period can be related to food intake.

Before employing the DLW technique, a preliminary study was carried out using isotope dilution (deuterium oxide) to measure the body water content and turnover rate of the animals (Paper III). The data thus obtained have provided the basis for the calculation of the dose of the considerably more expensive isotopes employed to measure energy expenditure (Paper IV). In all three studies it has been necessary to chemically restrain the study subjects (Paper I and II). Finally, results from the direct observations of feeding and from the isotope dilution experiment were utilized for an estimate of the total impact of walruses on the main bivalve species in Young Sound (Paper VI).

Figure 4 Seal remains (skin and hair) from the excrement of a walrus at the Lille Snenæs haulout in August 2000. An Arctic fox (*Alopex lagopus*) was observed scavenging on these remains at the haulout site, uncaring of the presence of walruses (photo: M.Acquarone).

Studies of walrus feeding in the wild

Etorphine immobilization

Any procedure requiring contact with the study animals necessitates a reliable immobilization technique to enhance the researchers' safety, to speed up handling and to reduce handling-induced stress on the animal. For small and non-aggressive pinnipeds and for procedures, which require brief handling, manual restraint is usually sufficient. E.g. the animals are netted or a bag is lowered on their head (Stirling 1966).

A chemical immobilizing agent can be administered directly or prior to manual restraint and it is preferred in the case of larger or aggressive pinnipeds. According to the species and the field condition it is possible to choose among several delivery techniques and drug types. In some cases, usually where logistics is not a problem, it is possible to employ inhalation techniques which have the advantage of being safe for the animals and of being a controllable restraint procedure (Stirling 1966; Heard and Beusse 1993; Heath et al. 1996; Heath et al. 1997). One of the drawbacks of using a gas anesthetic is that it requires a bulky apparatus, which renders it impractical for use in very remote stations where transport involves flying. Furthermore, it is not feasible to use this technique on an aggressive and potentially dangerous animal like a walrus.

The portability of the equipment required for the intramuscular delivery of a liquid agent and the availability of potent drugs allowing rapid immobilization with short induction times are valuable aspects for the immobilization of walrus. Most important the delivery of a small amount of drug increases the accuracy the technique allowing the researcher to pick an individual animal out of a group. For this species the typical apparatus consists either of an injection syringe on a jabstick (Stirling and Sjare 1988) or a pressurized dart and a delivery system consisting of an air or powder gun (DeMaster et al. 1981; Born and Knutsen 1992a; Griffiths et al. 1993; Lanthier et al. 1999). Several drugs have been employed for the immobilization of walruses (for an overview, see introduction to Paper I). For this study the choice of drug was dictated by several criteria among which:

- very rapid induction time, to reduce the risk of the animal escaping to sea before full immobilization (at both haulouts the walruses typically lay less than 10 m from the shoreline)
- safety, for the researchers during attachment of equipment to the tusks.
- repeatability, because of the necessity to recapture a specific animal during the isotope dilution experiments
- possibility of extending the immobilization time for repeated blood sampling during the isotope dilution experiments

The combination of etorphine/diprenorphine was chosen because it met the above criteria and because of the extensive experience of the participants in this study (E.W.Born and D. Griffiths) in using these drugs for walrus immobilization in the wild. The immobilizations performed in the course of this thesis work have provided valuable additional data on the chemical restraint of walruses by etorphine/diprenorphine. Thus data from 33 immobilizations by etorphine HCl, performed between 1999 and 2001, was added to the 36 immobilizations previously performed by Norwegian and Danish researchers between 1989 and 1994 and has provided the basis for Paper I which describes and evaluates this chemical restraint technique.

From the overall experience summarized in Paper I it appears that etorphine/diprenorphine is an effective method for the rapid immobilization of free-living walruses. The mean induction time of 5 min reported in this work is acceptable for most studies of walruses hauled out on land. The delivery of the drug can be effectively performed without scaring the animals out to sea. After complete immobilization it is possible for the researchers to work on the animal at least for an average of 14 min corresponding to the etorphine-induced apnea. This period can be effectively utilized for procedures involving the most intense handling, instrument attachment to the tusks, insertion of flipper-tags and any other handling requiring intense manipulation of the animal (e.g. weighing, blood sampling, catheter insertion).

From the data available it has been possible to estimate the overall mortality rate of etorphine immobilization to 7.3%. Also worth noticing is that in the course of this work it has been necessary to recapture the animals for up to 4 times within the same field season. During recaptures there has been no mortality, which indicates that the overall mortality could actually be due to individual hypersensitivity to the drug. The recaptured animals did not show any significant negative effect from the repeated medications and handling.

Medetomidine immobilization

Though etorphine/diprenorphine immobilization provides sufficiently long restraint of the animals for non-invasive procedures, it is not an adequate method for prolonged immobilization of specific individuals, for procedures requiring prolonged handling such as blood sampling. Additional drugging is required in these situations. A medetomidine-ketamine combination administered after immobilization by etorphine/diprenorphine was used during the only long-term immobilization of free ranging walrus reported in the literature (Lydersen et al. 1992). The combination of the alpha-antagonist medetomidine with other drugs is regularly used for the immobilization of domestic or captive (Greene 2003; Portas et al. 2003; Miller et al. 2003) and free-ranging animals (Spelman et al. 1994; Fournier et al. 1998; Fournier-Chambrillon et al. 2000; Walsh and Wilson 2002) among other reasons because of its safety for humans as it is not easily absorbed through the skin in case of accidental contact. Medetomidine with zolazepam (Telazol) has also been previously used for the immobilization of captive pinnipeds (otariids and phocids) and has produced a smooth induction and a spontaneous recovery (Roken B. 1997). This combination has been successfully used for walruses in Canada since 1998 (R.E.A. Stewart *via* E.W.Born *pers. comm.*), but has proven fatal for two walruses in NE Greenland (Griffiths unpublished). Suspecting that the lethal effect for the animals in Greenland was due to zolazepam, E.W.Born suggested the use of medetomidine alone.

From the experience gathered in this thesis (Paper II), medetomidine has proven suitable for the prolonged restraint of adult male walruses (1,000-1,500 kg) for time-consuming procedures, after immobilization with etorphine/diprenorphine. Medetomidine should be administered after reversal of etorphine by diprenorphine and full regaining of respiratory function. The drug should not be used intravenously as it immediately induces apnea even in very small doses. Intramuscular administration worked best in small 10-20 mg increments until the breathing rate reaches approximately 1 breath per min. The animals in this study were kept restrained and manageable for up to 6.75 hr which has allowed the performing of isotope infusion and repeated blood sampling (see next section and Paper III and IV).

Isotope dilution studies

To investigate walrus food consumption in the wild it has been necessary to adopt two different approaches:

- Measures of Field Metabolic Rate (FMR) by the DLW technique to estimate the integrated energetic requirements of two individual walruses over a period of time of up to 11 days (Paper IV), and
- Direct observation of walrus feeding to determine meal composition, meal size and meal frequency by direct observation and indirectly by satellite telemetry (Paper V)

However, due to the prohibitive cost of DLW, to minimize the dose, and consequently the costs, of the DLW for the FMR study, it has been necessary to investigate the size of the body water pool and the magnitude of body water turnover of the study animals. Both measures have been effectuated by hydrogen isotope dilution by deuterium oxide (Lifson et al. 1955; Speakman 1997). Paper III exposes the results of these measures with an indication of the magnitude of these two important parameters which also allowed an estimate of the body composition of the experimental subjects using allometric equations originally developed for gray seals (Reilly and Fedak 1990).

Body water content and body water turnover

Seven adult male walruses were enriched with an intravenous injection of deuterium oxide. The time required for the complete equilibration of deuterium in the body water pool was assessed through the analysis of the concentration of the isotope in repeated samples of venous blood, taken at regular intervals for up to 6 hr from enrichment. The sample corresponding to the time of complete equilibration was then used for calculating the size of the body water pool. Because only one attempt at a prolonged immobilization of a walrus was available from the literature (Lydersen et al. 1992), and because walruses are notoriously difficult to immobilize (Paper I), this procedure was crucial for the correct determination of the minimum immobilization time required for the studies involving isotope dilution (Paper III and IV). The results indicate that 2-3 hr from the enrichment time are necessary for complete equilibration. The value is considerably longer than the value of 1 hr reported by Lydersen et al (1992). Speakman (1997) argues that the surprisingly short time compared to the size of the animal reported by Lydersen et al (1992) is explainable by the fact that the animal was injected intravenously and that venous blood was subsequently sampled. However, although also in the present work the animals were also both enriched intravenously and sampled for venous blood, the observed equilibration time is considerably longer. In the case of intravenous injection and venous blood sampling before equilibration, an underestimate of the body water pool size will result, as the blood concentration of the isotope will be higher than at equilibration. Conversely sampling after equilibration would result in lower blood isotope concentration and therefore overestimate pool size. However, it is preferable to sample too early rather than too late. By sampling too early any error in the dilution space will partly be compensated by the covariant error in the derived gradient (Speakman and Racey 1989) while at increasing time distance from enrichment, the derived dilution space is independent of the dilution curve (Speakman 1997).

The body water pool size was calculated to range 37-73% of total body mass (TBM) and the water turnover averaged 45 g/kg_{TBM}*day. These two parameters were used to determine the minimum initial dose of $H_2^{18}O$ for the DLW study. The amount of isotope required to enrich an animal with DLW depends on several considerations. As the largest pinnipeds reported to have been enriched with DLW weighed 114 kg (Costa and Gales 2003), and the largest terrestrial animal 107 kg (Haggarty et al. 1998), there were no indications of the requirements for the enrichment of animals the size of adult male walruses (typically over 1,000 kg). Therefore data obtained from the deuterium dilution study were utilized to back-calculate the expected enrichment dose.

Allometric equations derived for gray seals (Reilly and Fedak 1990) have been applied to the data on body water content. The body fat content of the measured walruses averaged 25% of TBM. This value has not been validated but it is not significantly different from the walrus-hunt data that report fat at 18% of TBM (Knutsen and Born 1994) and from the value of 21% of TBM obtained from weighing the blubber after dissection for one individual deceased during this study (Paper III). The calculated value for body protein (18% of TBM) could unfortunately not be compared, as studies on walrus reporting this value were not available. Likewise body ash and total body gross energy, for a 1,300 kg walrus were estimated respectively at 26 kg and 18,300 MJ.

Walrus Field Metabolic Rate by DLW

The metabolic energy requirements of an animal determine the quantity and quality of the diet, which has to provide metabolic fuel. In general the ingested energy and mass has to balance the energy and mass that is used for maintenance and activity and loss as waste products. Thus, a measure of the energy expended can be correlated to food intake and therefore to the impact the animal has on the ecosystem. One method for measuring the energetic requirements of a free-ranging animal is the DLW technique developed originally by Lifson and McClintock (1966) and described in detail by Speakman (1997). This method estimates CO_2 production over a period of time from which energy consumption can be derived provided the food quotient of the diet is known.

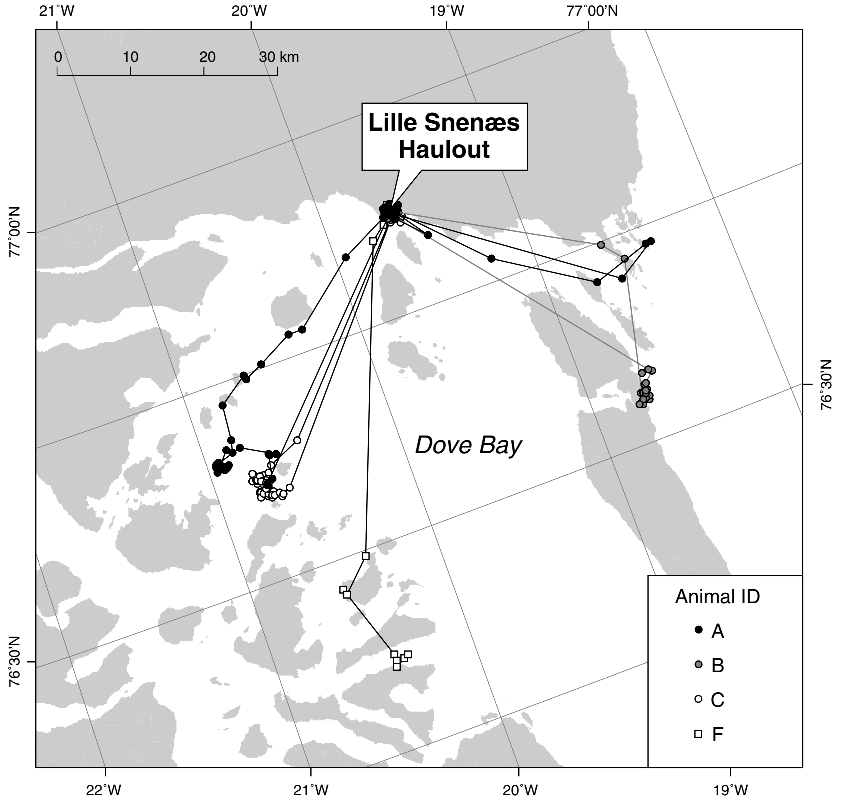


Figure 5 Satellite tracks of two animals that were enriched with DLW (A and B) in 2001 at the Lille Snenæs haultout and two other animals tracked during the same period (C and F). All four animals were equally active during the period (Paper IV).

CO_2 production measures in the DLW technique are based on the experimental observation that the oxygen in the CO_2 exhaled by an animal is in isotopic equilibrium with the oxygen of the body water. The decrease in time of the ^{18}O in the body of an animal enriched with $H_2{}^{18}O$ is related both to rate of CO_2 and to body water loss. Similarly the decrease in time of ^2H in an animal enriched with ^2H_2O is only related to the loss of body water. Thus, by enriching the animal with a mixture of ^2H_2O and $H_2{}^{18}O$ it is possible to monitor the turnover rates of both the hydrogen and oxygen isotopes. Finally, the difference between the hydrogen and the oxygen turnover rate it is proportional to the rate of production of CO_2 which is subsequently used to calculate O_2 and thus energy consumption.

The dose of DLW to be used for the enrichment of the animals was determined from the previous deuterium dilution study (Paper III) and injected intravenously. Venous blood was sampled for both the enriched animals for a total of 4 hr at short intervals. The isotope concentration in the blood was utilized to calculate the magnitude of the body water pool. Both animals were then allowed to regain the sea. Evidence from satellite tacking shows that the two animals have been normally active during the measurement period (Figure 5). After a period of respectively 5 and 9 days the animals returned to the beach at Lille Snenæs and were again sampled for blood. This allowed the calculation of the average CO_2 produced in the measurement period and therefore of the animals' Field Metabolic Rate (FMR) (Paper IV).

The values of 345 MJ/d for the 1,370 kg and 417 MJ/d for the 1,250 kg animal have been derived using the calculations proposed by Lifson and McClintock (1966) for the single pool model which have been employed for most other DLW studies of FMR pinnipeds. A two pool model (Speakman 1997), which is more appropriate for animals larger than 10 kg, would yield respectively in 328 MJ/d and 365 MJ/d probably reducing the risk of overestimation. These values correspond to over 6 times the predicted basal metabolic rate (BMR) for animals this size (Kleiber 1932; Kleiber 1961). Which is consistent with similar studies effectuated on other pinniped species. Validation studies of the DLW technique have demonstrated that the values of CO_2 production are correct within 10% (Nagy 1989).

The DLW technique is based on a range of assumptions the deviation from which can induce errors in the calculations. The possible errors have been discussed by Lifson and McClintock (1966), Nagy and Costa (1980), and Nagy (1980). Basically it is assumed that during the measurement period:

1. **The rates of CO_2 and water flux are constant.** But the errors are insignificant if the variations in flux are small (Nagy and Costa 1980) and they can be reduced by using a two-point sampling technique (Speakman and Racey 1986) which is used in this thesis.
2. **The relative concentration of the isotopes in the body is the same as in the products leaving the body.** That is to say that there is no fractionation or deposition of isotopes. The errors derived from the violation of this assumption are theoretically lower than 10% (Lif

son and McClintock 1966). Only a thorough validation study can assess the validity of this assumption.

3. **The isotopes are lost only as CO_2 and H_2O** as is the case with most mammals (Nagy and Costa 1980; Nagy 1980).
4. **No CO_2 or water enters the body through the skin or lungs.** Firstly ambient CO_2 levels in the air are low and therefore there is no reason to think this assumption is violated (Nagy 1980). Furthermore, In feeding pinnipeds the high water content in the diet minimizes the importance of violations of this assumption (Depocas et al. 1971; Costa 1982). For fasting animals it could potentially induce large errors as water flux decreases, but for animals living in cold climates the relatively low water content of the air should limit this error.
5. **The isotopes label only and completely the body H_2O.** It is a rare phenomenon (Nagy 1980), but deviations from this assumption cause an error in the estimation of total body water (TBW) (Culebras et al. 1977; Nagy and Costa 1980) that can be reduced by critically utilizing both ^2H and ^{18}O dilution in the calculation of TBW as ^{18}O is likely to be a better estimator of TBW (Nagy 1980; Schoeller et al. 1980).
6. **The animal's body water remains constant.** Which is difficult to control unless TBW is measured both at the beginning and at the end of the measurement period. However, Nagy (1980) demonstrated that a 50% variation in TBW only induces 5% error in the calculation of CO_2 production.

In the study presented in Paper IV assumptions 1 through 4 are satisfactorily met. For assumption 5, the effect of the incorrect body water pool size due to the use of deuterium as a tracer has been minimized by utilizing the mean dilution space ratio in the calculations (Schoeller et al. 1986) while the two-pool model (Speakman 1997) accounts for the differences in isotope diffusion velocity between the different body water compartments as expected for large animals. Finally, assumption 6 is difficult to verify as it has only been possible to re-determine the body water pool size at the end of the measurement for one of the experimental animals. For this walrus it was possible to estimate an increase in pool size by 18% over 20 days corresponding to the longest recapture interval in this study. Such an increase should not induce more than 5% errors in the estimate (Nagy 1980). It is therefore likely that all other estimates lie within this estimate error level.

Another potential error arises because, to derive energy expenditure from CO_2 production, it is necessary to calculate the respiratory quotient (RQ) of the diet (Kleiber 1961). In paper IV an RQ = 0.85 is used for the calculations. This is approximated from the composition of the lean mussel diet of the walruses in NE Greenland described in paper V and approximated to the food quotient of the diet according to the method exposed in Black et al. (1986). The RQ of typical marine mammal diets lies in the range 0.74-0.77 (Costa 1987; Boyd et al. 1995). However, it has been reported that the RQ of gray seals vary considerably throughout the day suggesting these seals can rapidly change metabolic fuel as they apparently enter a starvation state already after 24 hr of food deprivation (Boily and Lavigne 1995). Considering that walruses alternate feeding bouts to long haul out periods it is possible that they also regularly alternate the metabolic fuel. Therefore, the RQ derived from the diet might not be accurate enough as an average value for the measurement period.

In conclusion the estimate of FMR by the DLW technique in Paper IV is comparable with similar studies of pinnipeds. The high value of energy expenditure compared to the BMR predicted by the Kleiber (1961) equation is within the limit of the observed maximum rate of energy expenditure for warm blooded animals (Hammond and Diamond 1997) and is probably due to concurrent factors including high activity, low ambient temperature and moult period. The energy values obtained from the FMR measures, when converted to "mussel-equivalents" correspond to 67 kg wet-weight per day (5% of TBM) for the 1370 kg walrus and 75 wet-weight per day (6% of TBM) for the 1250 kg walrus. These values are plausible when considering that a food intake of 5-7% of TBM has been estimated by Fay (1982), while 4.7% has been calculated from direct underwater observations of feeding walruses in the wild (Paper V).

For adult animals in captivity, with low activity levels and maintaining body weight, Kastelein et al (2000) have measured an average food intake of 1.8-3.1% of TBM. Fisher et al. (1992) even report a food intake for a high-energy diet of corresponding to 1.3-1.8 % of TBM, however all but one of the animals in that study lost weight. The difference between the food intake of free-living and captive walruses is striking. It could only reasonably be ascribed to the differences in activity and physical setting between the two conditions (Paper V) or, since the measures in paper V are made during the summer, to seasonal variations in food intake (Kastelein et al. 2000).

Direct observations of feeding walruses

A study involving the direct underwater observation of feeding was set up as an alternate way of obtaining estimates of food consumption in the Young Sound area for walruses utilizing the Sandøen haulout (Paper V). Average meal size, composition and frequency were investigated by coupling systematic observations of feeding walruses with satellite telemetry measures of area use and diving behavior with underwater monitoring and examination of the remainders of the meals (bivalve shells).

The animals were monitored from an observation post on the shore to obtain an indication of dive frequency and area use involving theodolite triangulation. When possible a diver on stand-by would descend to the feeding patch and video-record the feeding walrus. After the meal the divers would collect the empty shells from the feeding furrow. After sorting out old shells from the freshly eaten it was therefore possible to obtain both qualitative and quantitative data on meal size and composition (Figure 6). These data were then coupled with a chemical analysis of the energy content of the mussels which contributed to the estimate of the nutritional levels of the meals and with behavioral data on haulout and dive activity to estimate meal frequency.

Figure 6 Freshly eaten empty shells gathered from a foraging patch after a walrus visit. From top-left to bottom right *Hiatella* sp., *Mya* sp. and *Serripes* sp. This tray represents the bivalve food intake of one dive (photo S. Rysgaard & M. K. Sejr).

It was recorded that walruses dived mainly to depths of less than 32 m. Dives would last for an average 6.7 min with 1.0 min ventilation interval at the surface, resulting in submersion for 87% of the time at sea. During the feeding dives an animal of 1230 kg TBM ingested an average of 583 g of shell-free wet-weight corresponding to 2.6 MJ per dive. It is also interesting to note that the animals mean meal was composed, in dry weight, of *Mya* sp. (82%), *Hiatella* sp. (4%) and *Serripes* sp. (14%) which is in accordance with previous observations on the specific composition of walrus meal (Vibe 1950; Fay 1982; Fay et al. 1984; Fisher and Stewart 1997; Sheffield et al. 2001). For the calculations in this study it was assumed for all species that the whole bivalve (siphon and body) was consumed. This assumption is based on the observation that less than 3% of the mollusk is left in the shell after predation by a walrus in Young Sound (Paper V). In northern Foxe Basin though, Fisher and Stewart (1997) observed that only the siphons of *Mya* sp. and *Hiatella* sp. were consumed by walruses, while for *Serripes* sp. both body and siphons were found in some of the stomach contents they analyzed. Fisher and Stewart (1997) point out that the ability of walrus to consume whole clams depends on the behavior of the clams and on the substrate in which they reside. By integrating these data with observations of haulout and diving activity it was possible to estimate a daily feeding rate of 4.7% of TBM. These independently obtained results (Paper V) confirm the estimate of FMR obtained by the DLW technique for walruses of similar size. It is worth noticing that this calculation involves only the bivalve portion of the benthic community and that it is necessarily a conservative estimate of meal size and composition. The macrozoobenthic biomass of Young Sound at depth not deeper that 32 meters other than bivalves is also composed of brittle stars, crinoids, holoturians, polychaetes and other mollusks (Sejr et al. 2000) which are thought to be a regular component of walrus diet as well (Fay 1982).

Impact on the Young Sound ecosystem

The results of the investigations on feeding (Paper IV and V) integrated with area use data from satellite telemetry of walruses tracked in Young Sound, on group size derived from visual observations and genetic identification of individuals are summarized in paper VI.

This study presents a picture of the magnitude of the ecological significance of walruses in the Young Sound inshore area. The mussel banks in Young Sound are abundant along its northern shore (Sejr et al. 2000) and the walruses feed on these mollusks down to a depth of 40 m defining an area of about 33 km^2 suitable for walrus feeding in Young Sound. From direct observations and genetic identification it has been possible to define that the walruses hauling out on Sandøen island, at the fjord's mouth, number circa 40 adult individuals. Satellite tracking data reveal that during the 90 days long open water season these animals spend on average 44% of their time in the inshore Young Sound. Approximately 30% of this time is spent in the water inside of the study area. Considering a TBM of 976 kg for the average walrus in the area, and a daily feeding rate of 6-7% of TBM, walrus predation on the bivalve population amounts to 63-74 tons shell-free wet weight per open water season. This amount corresponds to 1.2-1.3% of the standing biomass of *Mya* sp. and *Hiatella* sp. and represents 19-28% of the annual production for these species. Similarly, from detailed diving activity data, selecting only what appears to be feeding dives (below 6 m and with a duration of 5-7 min) walruses attain a total consumption of 74-114 tons shell-free wet weight per open water season. This value probably represents an overestimation because not necessarily all dives selected with this criterion represent feeding dives.

Furthermore, walruses can both influence the structure of the benthic communities and contribute to the recycling of nutrients by sea-bottom tilling that releases nutrients trapped in the sediments (Oliver et al. 1983; Fukuyama and Oliver 1985; Oliver et al. 1985) just as gray whales (Oliver and Kvitek 1984; Oliver et al. 1984; Oliver and Slattery 1985; Kvitek and Oliver 1986; Nelson et al. 1987; Nelson and Johnson 1987). This increase in nutrient flux between the sea bottom and the water could increase the productivity of the fjord ecosystem, and specifically of the bivalve communities, partially counteracting the increased predation pressure by the walruses.

General considerations

Immobilization by etorphine

The immobilization of free-ranging walruses is difficult, and there does not seem to be any ideal drug at the moment. Etorphine is dangerous for humans and for that reason has been withdrawn from many markets (e.g. the US) making other drugs the only viable alternative for people operating on those territories. While some of these alternatives are being used with promising results, data is still to scarce to state that a valid substitute to etorphine has been found. A workshop on walrus immobilization, held in San Diego (CA, USA) in February 2002 (Anon. 2002), gathered some experienced 15 walrus researchers. The meeting provided a forum for the exchange of information, directions and some considerations on future attempts, but failed to come up with a universal solution. The complexity of the reactions of walruses to narcotics has been underlined and a range of alternative methods has been presented. Unfortunately it is not feasible to research intensively on walrus immobilization and there does not seem to be any alternative animal model for large scale testing. As there is no designated culprit for the mortalities listed in Paper I it is important to keep in mind that individual hypersensitivity might be unavoidable for some drug types. A mortality rate of 7% is certainly very high when working on highly endangered populations. However, until the beginning of this thesis work in summer 2000, etorphine immobilization was generally considered safe for the animals. The available data included 26 immobilizations and only one casualty (possibly explained by renarcotization due to the incorrect administration of diprenorphine). It is here suggested, when possible, to initiate the immobilization procedure with a shot of pre-anesthetic drug to prepare the designated animal to the delivery of the primary drug and help standardize the immobilization conditions among the different animals. Most of the animals in this study only slightly reacted to darting indicating a high tolerance to the firing noise and the dart impact, making it feasible to prepare and deliver two consecutive darts at a short interval. The need for careful and complete monitoring of the whole immobilization procedure and of the reactions and the physical/physiological parameters of the animal cannot be overemphasized.

Immobilization by medetomidine

The action of medetomidine to prolong the immobilization period is satisfactory provided that the drug is delivered intramuscularly. It more that doubles the time available for working with the animals, and is readily reversed by its antagonist. Possibly the limits to this technique lie in the consequences of keeping a walrus immobile for a long period of time, including body temperature rise, accumulation of mucus in the lungs and the airways and mechanical impairment of full respiratory function caused by the animal's own weight on it's chest. Respiration rate, body temperature and heart rate have to be monitored carefully. In paper I some data on blood pH during immobilization are presented. During immobilization the animals can go from blood acidosis during the drug induced apnea to alkalosis while hyperventilating in the recovery phase. Even though pinnipeds evolutionarily must be highly tolerant to low values of blood pH, it is clear that this is a parameter worth monitoring in the course of future immobilizations.

Water metabolism by isotope dilution

The study of water metabolism through isotope dilution has provided novel insight into the physiology of walruses. The data on water metabolism could under certain conditions be utilized for the calculation of food intake or fasting metabolic rate (Costa 1987). Unfortunately, in the case of this study, essential parameters as the metabolic efficiency of the diet and the amount of metabolic water produced per unit food metabolized were not available. These can profitably be the subjects of future work. Apparently body composition, and in particular body fat, can be easily calculated in this species utilizing the allometric equations developed for gray seals (Reilly and Fedak 1990). This study, requiring only one immobilization, can be extended in the future to a larger number of individuals to provide a picture of the body condition of the population. By repeating this analysis on the same individuals both at the beginning and at the end of the feeding season it would be possible to measure their fattening rate and thus provide an important ecological parameter for the understanding of their life strategy.

Energy metabolism by DLW

Measures of pinniped FMR by the DLW method span, with this study, over a wide size range (27-1,300 kg). Although they are not all performed under the same conditions it is irrefutable that their results are consistent with each other. The regression line that best describes the relationship between pinniped body size and FMR (Paper IV, fig 2) unmistakably shows metabolic rates 5-6 times the predicted BMR from the Kleiber equation (Kleiber 1932; Kleiber 1961). Lavigne et al. (1986) convincingly argument that the BMR of marine mammals is not larger than that of terrestrial mammals of similar size, which leads to speculations about the reason for this large difference between the metabolic rate of resting and active animals. However, it is also very peculiar that the metabolic rate of active pinnipeds, measured with other techniques than DLW, under experimental conditions only attains 3 x BMR (e.g. in Boyd et al. 1993; Hurley and Costa 2001). Furthermore, the maximal sustained metabolic rates of pinnipeds under aerobic metabolism have been measured at 6-7 x BMR (Elsner 1987; Butler et al. 1992) suggests that pinnipeds FMR is close to the limit for sustained workload (Hammond and Diamond 1997). It is difficult to think that an animal would live at its maximum metabolic rate for more than a short period of time and surely not weeks as indicated by the DLW studies. Nonetheless the DLW technique infers energy expenditure through measures of CO_2 production. There are some indications that DLW might overestimate CO_2 production by up to 36% (Boyd et al. 1995) compared to respirometry measures. This discrepancy underlines the necessity of validation studies comparing different methods of estimating FMR. The ability of pinnipeds to work for longer periods under anaerobic conditions might induce errors in the measure of CO_2 production by respirometry, usually assumed as the standard, by inducing a delay between the use of metabolic fuel and the exhalation of CO_2. Some studies have employed heart rate in parallel with DLW to estimate FMR in pinnipeds (Costa et al. 1989; Arnould et al. 1996; Boyd et al. 1999). Estimates by heart rate lie 30-40% below the DLW measures and seem to agree with respirometry data, which calls for extensive cross-validation of the different techniques available.

The estimate of FMR in this work is based only on two animals, but the sheer cost of the DLW did not allow for more animals to be enriched with the same grant. Similarly, the activity levels of the two individuals, measured by satellite telemetry and dive recorders, have been measured simultaneously with their metabolic rate, the data are not sufficient to infer any statistical correlation between metabolic rate and activity levels. Furthermore, the study could have been improved by an additional enrichment and calculation of the body water pool size at the end of the measurement period, possibly supplemented by the determination of body mass by weighing at both captures. The weighing of the animals has been attempted earnestly in this work, but three men could not roll a walrus over the rocks of the extremity of the haulout and keep a half-drugged walrus on the weighing apparatus (Figure 7). Unfortunately other weighing systems could not be transported to the location.

Figure 7 Weighing system used in NE Greenland 2000-2001. The immobilized walrus is rolled onto an aluminum rack resting on 4 load cells (photo: M.Acquarone).

Observations of walrus feeding

The difference between the freshly eaten bivalve shells left on the sea bottom from those from previous meals is unequivocal (Sejr 2002, and Rysgaard and Sejr pers. comm.). The number and species of mollusks consumed during dives is therefore thought to be quite reliable. However, it is important to keep in mind that the benthic community includes more than bivalve mollusks and that walruses might predate on the other components too. And, even if the animals did not seem to be affected by the divers accompanying them to the feeding patch, the disturbance due to the high activity levels in the area (motorized inflatable dinghies, airplanes etc.) could possibly reduce foraging activity and make the calculation of food consumption in Paper V an underestimate of the actual food requirements. Nonetheless, the direct underwater observation of walrus feeding (Paper V), which provides an untraditional insight into meal size and meal composition, reveals a similar value for food consumption compared with measures derived from FMR by the DLW technique.

Estimates of predation

The values for walrus food consumption derived by direct observation (Paper V) have been used to estimate walrus predation on bivalves in the Young Sound area (Paper VI). These are in accordance with those that can be derived from measures of FMR, which confirms the validity of the estimate. However, it is important to keep in mind that walrus diet is likely to be composed of more than the two principal bivalve species. Furthermore, it is possible that the location designated as the walrus feeding ground actually is not the only one in the fjord, even though bathymetry direct observation and track data all seem to point in that direction. These factors indicate that the impact of walruses on the bivalve population (Paper VI) is a conservative estimate.

Concluding remarks

The novel observations about the feeding requirements of walruses presented in this thesis have answered some questions, but have also opened the way to further research on the ecological significance of these animals in the arctic fjord ecosystem and on the status of the walrus population in NE Greenland. Some preliminary study on the trophic relations between the different ecosystem components using stable isotopes (Hobson and Welch 1992) has already been carried out in Young Sound (Sejr 2002). The results from this investigation have placed the walruses at a similar trophic level as *Sclerocrangon boreas* and *Buccinum* sp., both scavenging or preying on bivalves, while common eiders (*Somateria mollissima*) appear to belong to a higher trophic level, presumably because they prey both on bivalves and on predators of bivalves such as amphipods (Weslawski et al. 1997). Therefore it is important to quantify the relative abundance of the possible walrus prey items, both bivalves and others such as holoturians, brittle stars etc. This investigation can be carried out by analyzing fatty acid profiles of the walruses and the potential prey species (e.g. see Dahl et al. 2003 and references herein). Samples of walrus skin and blubber can easily be obtained from the hauled out animals with a dart fired from a crossbow at a reasonable distance, which does not induce disturbance to the animals. This system has been successfully employed on Sandøen (2002-2003) to obtain samples for individual recognition through genetic identification (E.W. Born *pers. communication*). Samples from the prey items can be gathered in the course of the ongoing marine monitoring process in the area (Rysgaard et al. 2003a). The above mentioned genetic identification research program is important both for the definition of the size of the group of walruses utilizing the haulout and for the identification of family relationships among the group members and with neighboring groups (W Greenland, Svalbard) (Andersen et al. 1998; Born et al. 2001). Furthermore, an extensive deuterium dilution study performed with double enrichments at the beginning and at the end of the feeding season could, at a relatively low cost, provide information on the physical conditions and of the rate of energy acquisition for the local animals. The latter, supplemented by an investigation on the metabolic efficiency of the diet, could also be used to calculate food intake on a larger scale than in this work.

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Paper I: Evaluation of etorphine HCl reversed by diprenorphine HCl for immobilization of free-ranging Atlantic walrus (*Odobenus rosmarus rosmarus* L.)

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Abstract

To date no standard method exists for the immobilization of free-ranging walruses (*Odobenus rosmarus*). In the period 1989-2001 a total of 69 immobilizations with etorphine HCl were performed by remote darting on 41 individual free-ranging adult Atlantic walruses (*O. r. rosmarus*), with body masses 633 - 1883 kg, as a prerequisite for the attachment of radio tracking and dive recording instruments and for studies of metabolism. Ten individuals were immobilized several times. Data on these immobilizations are presented and the method is evaluated. Full immobilization was achieved in 58 cases (84%). The animals were insufficiently restrained in 6 cases (9%) and 5 animals died (7%) following the immobilization. The animals were fully immobilized and approachable after 5 min (n = 38, range 1.9 - 12.4 min, SD = 2.2) with a dose of etorphine of 6.10 µg/kg (range 2.4 - 12.6 µg/kg, SD = 2.4). Induction time was negatively correlated with the dosage of etorphine. Etorphine-induced apnea lasted 13.7 min (n = 36, range 17.0 - 26.7 min, SD = 5.1) and was reversed by multiple doses of the antagonist diprenorphine HCl. The first dose of antagonist of 12.2 mg (n = 39, range 6.0 - 21.0 mg, SD = 3.5) was administered 8.4 (n = 38, range 4.7 - 18.0 min, SD = 2.8) min after injection of the agonist. Total dose of diprenorphine per animal ranged between 7.7 and 41.7 µg/kg (n = 31, *mean* = 17.2 µg/kg, SD = 7.5). Following apnea the animals experienced low blood pH values (min pH 6.8). For animals that were immobilized several times there were no indications of changed sensitivity to etorphine as reflected in unchanged induction times. Mortalities could neither be related to the doses of agonist and antagonist, or to the times of administration of the drugs. From this (n = 69) and other (n = 103) studies reporting etorphine immobilization of walruses (both Atlantic and Pacific) the overall success rate is 83% (8% casualty rate). It is concluded that the etorphine-diprenorphine combination is suitable for both single and multiple immobilization of walruses provided that (a) the antagonist diprenorphine is administered fast and well into a tissue with good blood irrigation, and (b) the animal is promptly intubated endotracheally to secure regain of breath.

Keywords: walrus, *Odobenus rosmarus*, immobilization, chemical-restraint, etorphine, diprenorphine.

Introduction

Chemical immobilization is usually necessary in studies of pinnipeds that require contact with the animals for e.g. attachment of instruments or physiology experiments. This is in particular the case when working in the wild with large and potentially dangerous species like walrus (*Odobenus rosmarus*). Captive and free-ranging Atlantic (*O. r. rosmarus*) and Pacific (*O. r. divergens*) walruses have been occasionally immobilized for surgery or for research purposes using a number of different protocols and drugs in the quest of a reliable method that is safe to the animals and the researchers. A series of anesthetic agonist agents have been employed, alone or in combinations, for surgery of captive walrus such as ketamine (Hagenbeck et al. 1975) or meperidine sulfate and thiamylal (Cornell and Antrim 1987). On freeranging walruses several drugs, alone or in combinations, have been used: phencyclidine and acepromazine (DeMaster et al. 1981), tiletamine and zolazepam (Stirling and Sjare 1988; Hills 1992; Griffiths et al. 1993), medetomidine and ketamine (Lydersen et al. 1992), etorphine (Hills 1992; Born and Knutsen 1992a; Griffiths et al. 1993), carfentanil (Hills 1992; Lanthier et al. 1999) (Table 1). However, the description of the effects and the response of the animals to the different drugs is fragmentary and more importantly none has proven optimal for this species.

Table 1 Summary of the immobilizations of walruses reported in the literature and in the present study. The drug employed and the number of attempts, of partial immobilizations and casualties is here reported together with an indication of the geographical area and the bibliographical reference for each group.

Drug	Attempts	Un-successful	Casualties	Location (Ref.)
Ketamine	1			Captivity (Hagenbeck et al. 1975)
Ketamine	1	1		Alaska (DeMaster et al. 1981)
Phencyclidine	1	1		Alaska (DeMaster et al. 1981)
Phencyclidine and Acepromazine	7	2	2	Alaska (DeMaster et al. 1981)
Meperedine Sulfate and Thiamidal	1			Captivity (Cornell and Antrim 1987)
Tiletamine and Zolazepam	10	3	1	Canada (Stirling and Sjare 1988)
	7	1	3	Alaska (Hills 1992)
	3		1	Svalbard (Griffiths et al. 1993)
Medetomidine and Ketamine	1			Svalbard (Lydersen et al. 1992)
Carfentanil	4			Alaska (Hills 1992)
	6			Canada (Lanthier et al. 1999)
Etorphine	59	8	5	Alaska (Hills 1992)
	38	2	1	Svalbard (Griffiths et al. 1993)
	6		2	Svalbard (Griffiths unpublished)
	69	6	5	This study

Remote (by dart or extension syringe), intramuscular administration of immobilizing agents is preferred when working with large or dangerous species in the wild (Lynch et al. 1999). Only the potent synthetic opioids carfentanil and etorphine simultaneously satisfy the requirements of a minimum injection volume, rapid induction and the existence of an antagonist to control the reversal of the effects of the agonist. The use of carfentanil alone and in combination with naltrexone has been described for only four Pacific walruses (Hills 1992) and for six Atlantic walruses (Lanthier et al. 1999). The paucity of data for carfentanil does not allow a thorough evaluation of this immobilizing agent, while the relatively more extensive use of etorphine (172 immobilizations) provides a good base for evaluation.

Etorphine antagonized with diprenorphine for immobilization of Pacific walrus was first used in 1988 in Alaska (Hills 1992), and in 1989 for Atlantic walrus in Greenland (Born and Knutsen 1990; Knutsen and Born 1994). In these and subsequent field studies of walrus (e.g. Griffiths et al. 1993) the number of animals treated per season have been relatively low. Therefore the accumulation of data for evaluation of the feasibility of using a particular drug for immobilization of walrus has been a slow process. Since 1989 a total of 69 new immobilizations of Atlantic walrus have been attempted in Greenland and at Franz Josef Land. Preliminary data from some of the early work have been partially presented in technical reports (Born and Knutsen 1990; Born and Knutsen 1992a; Knutsen 1993; Born and Wiig 1995), which are difficult to obtain. This paper summarizes and evaluates the experience acquired to date in the use of etorphine for the immobilization of freeranging Atlantic walruses.

Materials and methods

During the open water season in June-August 198992, August 1994, and July-August 19992001 a total of 69 immobilization attempts were performed using etorphine on 41 individual adult Atlantic walruses (37 males, 4 females). The animals were darted at different locations in Greenland (Kane Basin in NW Greenland, Young Sound and Dove Bay in NE Greenland) and Franz-Josef Land (Appolonoff, Kuhn, Hayes and Hooker Island) both on land and on ice floes. Ten out of the 37 males in this study were treated repeatedly both within the same season and in different years (Table 2).

Table 2: List of multiple immobilizations of the same individual walruses (ID) by etorphine/ diprenorphine in NE Greenland between 1989 and 2001. The figures represent the number of immobilizations of the same individual within the same summer season.

	Year				
ID	1989	1990	1999	2000	2001
1989-3	2	1		1	2
1989-4	1	1			
1999-1			1	1	1
2000-1				4	
2000-2				3	3
2000-3				2	
2000-4				3	
2000-6				3	2
2000-8				2	3
2001-9					2

Immobilization protocol

The effect of etorphine HCl was reversed by the antagonist diprenorphine HCl. The drug concentrations used for immobilization at Franz Josef Land were 9.8 mg/ml etorphine HCl and 12 mg/ml diprenorphine HCl (Cyprenorphine, C-Vet Ltd., Bury St. Edmunds, Suffolk, U.K.). In Greenland the concentrations were: 4 mg/ml (EtorfinVet. Pharmacia, Denmark) and 9.8 mg/ml ("M99" Vericore Ltd., Dundee, Scotland or Etorfin Vet., Pharmacia, Denmark) for etorphine HCl and 6 mg/ml (DiprenorfinVet. Pharmacia, Denmark) and 12 mg/ml ("M5050", 12 mg/ml, Vericore Ltd., Dundee, Scotland) for diprenorphine HCl respectively.

The animals did not receive any preanesthetic medication. The drug was usually delivered after the animals had settled down on the terrestrial haulout and were resting. The etorphine was delivered in 3 ml plastic darts equipped with a 10 cm-long needle (Vario dart, Telinject USA, 9316 Soledad Canyon Rd., Saugas, California 91350, U.S.A.) which were shot using a 11 mm-gauge CO_2 powered darting gun (Daninject, Børkop, Denmark, and Telinject, Römerberg, Germany). Diprenorphine was administered by hand-injection. Syringes with 2 mm-gauge and 8 or 10 cm-long needles were used to secure the deposition of the antagonist in the muscle tissue under the thick skin and blubber layer.

All times reported in this paper are relative to the time of injection of etorphine measured with a digital chronometer (Casio, Japan). Important events during the immobilization were:

a) **Induction time**: Time elapsed between impact of the dart (injection of the drug) and time of approach (i.e. the animal was unable to move and typically had stopped breathing 30-60 sec before approach).
b) **Apnea**: Time elapsed between breathing stop (estimated to 30 sec before approach if not directly observed) and first, deep breath.

The immobilization protocol used was initially developed in Greenland in 1989, adjusted in subsequent studies and was basically similar to the one described in Griffiths et al. (1993) and Lanthier et al. (1999).

Most animals were immobilized during afternoon and evening when walruses prefer to haul out (Born and Knutsen 1997). Furthermore, during the afternoon the herd of walruses was less tightly packed (Born and Knutsen 1990). It then became easier to select a suitable animal for darting (*i.e.* ideally an animal with tusks that were large enough to carry a satellite radio and that presented a calm behavior), thus causing a minimum of disturbance to the other animals. When working in the pack ice, animals that appeared relaxed and were not associated with calves and other subadults that, if frightened, might cause acute disturbance were preferred. Furthermore the floes used for hauling out had to be large enough for the handling of the drugged animal (floe size > 50 m^2). The darts were shot from a distance of 15 - 40 m. The firing position was chosen to ensure maximum penetration of the needle and optimal drug delivery in the muscular tissue. It was also attempted to hit a body location out of the animal's field of sight to avoid that the sight of the dart might induce a reaction in the darted subject or its neighbors. Dart impact sites during immobilization events were: in the upper dorsal region, 19; the lower dorsal region, 19; the flank, 11; ventral region, 4; a limb, 1; unaccounted for, 15.

Etorphine can induce respiratory depression (Alford et al. 1974), and in walrus drug-induced apnea is an unavoidable side-effect of the administration of etorphine (Griffiths et al. 1993). To minimize the duration of the apneic period and therefore the risk of death by suffocation, the antagonist diprenorphine was administered immediately after approach. The first dose of antagonist was either administered intramuscularly in the shoulder or lumbar region (n = 51), intravenously (n = 5) or in the highly vascularized tongue (n = 7) if muscle tonus in the jaw region did not prevent the mouth to be opened.

In 2000 and 2001 the walruses were subject to physiology studies that involved taking of repeated blood samples for and extended period of time while the animals were on land. In order to extend the period where the animals were tractable after they had regained regular breathing following the injection of diprenorphine, a total of 6 animals were given small amounts of medetomidine ("Domitor Forte", 10 mg/ml, Orion Pharma, Turku, Finland) (Griffiths et al. *this thesis*). The medetomidine mean delivery time was 45 min from the etorphine injection (n = 9, range 30 - 78 min, SD = 15). Physiology and behavioral data for these animals after the administration of medetomidine were excluded from the analysis of the characteristics of the recovery from immobilization by etorphine alone.

In 1999-2001 the animals were routinely intubated with an endotracheal tube (Cook Large Animal Veterinary Products, 38 mm internal diameter).

Animal monitoring during immobilization and recovery

In 23 cases breathing rate was recorded prior to immobilization. Breathing rate was also routinely recorded during immobilization.

Heart rate of immobilized animals was monitored with an electronic pulse meter (Exersentry, Respironics Instruments, Inc., Monroeville, Pennsylvania 15146, U.S.A.) equipped with four sensors connected to 6 cm-long needles inserted, as far as possible from each other, through the skin.

Internal temperature was measured in two places with an electronic thermometer (DM852, Ellab, Copenhagen, Denmark): a blunt probe was inserted approximately 20 cm in the rectum and a needle probe was inserted in a hind limb 2 – 4 cm below the surface of the skin.

In 1999-2001 a catheter (Becton-Dickinson Secalon-T, 2.0 mm * 160 mm, Medisinsk Utstyr AS, Oslo, Norway) was inserted into the epidural vertebral venous sinus (n = 43) which provided access for blood sampling and intravenous drug delivery.

In five animals blood pH-values were measured in the field by use of i-Stat Portable Clinical Analyzer (i-STAT Corporation, 104 Windsor Center Dr., East Windsor, NJ 08520, USA).

Standard and zoological body length and axillary girth were measured to estimate total body mass (TBM) according to methods in Born *et al.*(2003). For three walruses which were immobilized on pack ice, TBM was estimated by comparing tusk dimensions with a TBM-at-age curve established from walruses sampled from the Greenlandic subsistence catch in northwestern Greenland (Born unpublished data). In 8 cases estimates of TBM were not available.

Statistical analysis

Time and temperature data were log-transformed when necessary in order to obtain equality of variances and homoscedasticity assumed in parametric tests. The assumed significance level in all the tests was p = 0.05.

Differences between males and females for dose of etorphine administered, induction time, duration of apnea, antagonist injection time, antagonist first dose and time of first breath after induction using a Mann-Withney *U*-test. In this and subsequent tests on group ***A*** data we excluded an animal which had an unusually long induction time. In this walrus the dart only penetrated 4 cm into the animal and presumably the agonist was injected into the blubber.

The data were subsequently sorted into two groups: group ***A*** includes animals immobilized only once (31 individuals) and the first immobilization for animals treated more than once (10 individuals) giving a total of 41 immobilizations; and group ***B*** comprising only the animals that were subjected to repeated immobilizations (10 individuals, 38 immobilizations including the first).

On group ***A*,** linear regression analysis was employed to evaluate the effects of the dose of agonist on the induction time and the duration of apnea and of the time incurred between the administration of the agonist and the antagonist on the duration of apnea. The differences in pulse and body temperature between the apneic and post-apneic period were tested by use of unpaired *t*-tests.

The cumulative effect of consecutive immobilizations of the same individual within the same season (group ***B***) was investigated. The relative dose of etorphine on the log transformed induction time and log transformed apnea duration was tested with and without the effect of the interaction between the two independent variables (i.e. relative dose of etorphine and time for etorphine administration and time from the previous immobilization). Similarly, the cumulative effect of the time for diprenorphine administration was tested on the log transformed apnea duration with and without the interaction between the two independent variables (i.e. time between etorphine and diprenorphine administration and time from the previous immobilization). A spatial Gaussian correlation model for unequally spaced repeated measures including the random effect of the individual walrus (SAS® PROC MIX procedure) was used (Littel et al. 1996) for this purpose.

Causes of mortality were investigated by bootstrapping 100 times and tested against etorphine dosage, diprenorphine dosage and time of delivery with respect to etorphine administration. Chi-square tests were employed to test the effect of the researcher in charge on the failure and mortality rate.

The statistical packages StatView® (version 5.0.1, SAS Institute Inc.) and SAS® (version 8.2, SAS Institute Inc.) were employed for data analysis.

Results

Environmental conditions

In Greenland immobilizations were performed on calm (n = 54, *mean* = 2.3 m/s, range 0 – 12 m/s, SD = 2.1) and relatively warm days (n = 54, *mean* = 4.9°C, range –1.0 - 12.3°C, SD = 2.8); at Franz Josef Land it was usually calm (0 – 6 m/s) and temperatures ranged between –8°C and +3°C.

Improvement of the immobilization protocol

In contrast to Griffiths et al. (1993) and Lanthier et al. (1999) and the early seasons described in this study, in 1999-2001 the walruses were routinely intubated to prevent asphyxia due to etorphine-induced muscle contractions around the airways. This operation was usually initiated relatively late during apnea. Due to contraction of the muscles around the jaw, glottis and trachea the tube was sometimes difficult to insert and required forced opening of the glottis. However, intubation proved useful to help the animals regain full ventilation.

Differences between males and females

There was no significant difference in the relative dose of etorphine administered to males and females (Mann-Withney *U*-test, p = 0.06). Likewise no significant difference between males and females was observed for apnea start (Mann-Withney *U*-test, p = 0.11), apnea duration (Mann-Withney *U*-test, p = 0.43), time of first injection of diprenorphine (Mann-Withney *U*-test, p = 0.79), amount of diprenorphine administered as a first dose (Mann-Withney *U*-test, p = 0.42) or time of first breath after etorphine administration (Mann-Withney *U*-test, p = 0.08).

First time immobilizations (group A)

The estimated total body mass of the animals in group *A* ranged from 633 to 1883 kg (Table 3). A total of between 4 and 10 mg etorphine was administered per individual walrus at an estimated mean dose of 6.0 µg/kg (Table 3). The higher the etorphine dose the shorter was the induction time ("log-induction time in min" = 1.068 - 0.55 * "etorphine dose in µ/TBM in kg", R^2 = 0.275, p = 0.004, n = 28).

Apnea was induced in all animals at a mean of 5.0 min after injection of etorphine (Table 3). Apnea lasted 13.7 min (Table 3). The antagonist diprenorphine was administered 8.4 min after darting (Table 3) at a mean first dose of 12.2 mg (n = 39, range 6.0 - 21.0 mg, SD = 3.5). The total dose of diprenorphine delivered to the animals that were successfully immobilized ranged from 7.7 to 41.7 µg/kg (Table 3). For the same individuals the recovery phase, beginning from the first breathing act after the apneic period, started between 11.5 and 34.0 min from the injection of etorphine (n = 30, *mean* = 18.7 min, SD = 6.0).

The duration of apnea and the dose of etorphine were apparently not correlated ("log-duration of apnea in min" = 1.211 - 0.136 * "log-µg/kg etorphine", R^2 = 0.03, p = 0.40, n = 27). However, if the antagonist was injected relatively late the apneic period became longer. There was significant positive correlation between the time of injection of the first dose of antagonist and the duration of apnea ("log-duration of apnea in min" = 0.697 - 0.451 * "log-time of injection of antagonist in min", R^2 = 0.20, p = 0.011, n = 33).

Respiration rate of undisturbed resting walruses was 3.9 breaths/minute (n = 23, SD = 0.82). The breathing rate following apnea was 9 breaths/min (n = 29, range 1 – 32 breaths/minute, SD = 6) up to 30 min from induction. During the following 175 min, the average breathing rate decreased (breath/min = 17.34 - 2.48 * "log-min after induction"; R^2 = 0.094). In particular, after 60 min from induction it was 6 breaths/min (n = 16, range 2 – 20 breaths/minute, SD = 4).

Table 3 Summary of first-time immobilizations (group *A*) presented in this study. "Induction time" represents the time between etorphine administration and complete immobilization. "Diprenorphine administered" represents the total dose of diprenorphine administered. And "Time for diprenorphine administration" represents the time for the first dose.

	Body mass (kg)	Etorphine administered (µg/kg)	Induction time (min)	Diprenorphine administered (µg/kg)	Time for Diprenorphine administration (min)	Duration of apnea (min)
Mean	1310	6.0	5.0	17.2	8.4	13.7
Range	633-1883	2.4-12.6	1.9-12.4	7.7-41.7	4.7-18.0	17.0-26.7
SD	294	2.4	2.2	7.5	2.8	5.1
n	34	33	38	31	38	36

Table 4 Summary of multiple-time immobilizations (group *B*) of 10 walruses presented in this study. Dose and time data include all immobilizations where data were available. "Induction time" represents the time between etorphine administration and complete immobilization. "Diprenorphine administered" represents the total dose of diprenorphine administered. And "Time for diprenorphine administration" represents the time for the first dose.

	Body mass (kg)	Etorphine administered (µg/kg)	Induction time (min)	Diprenorphine administered (µg/kg)	Time for Diprenorphine administration (min)	Duration of apnea (min)
Mean	1220	6.4	4.1	15.6	7.8	14.6
Range	1050-1600	3.4-9.3	0.9-9.0	7.7-32.2	2.9-13.1	4.8-35.2
SD	177	1.9	1.7	7.0	2.2	5.3
n	10	36	36	35	35	36

The pulse of one undisturbed animal before it received a dose of etorphine was observed from movements of the ventral skin to 55 beats/min (similarly two other hauled-out animals, not subsequently drugged, showed a pulse of 48 and 52 beats/min respectively). After drug delivery, during the apneic phase the pulse of this animal had risen to 60 beats/min. For all animals (n = 10) pulse rate during apnea averaged 54 beats/min (range 40 – 80 beats/min, SD = 13.6). Generally there was no significant difference (*t*-test, t = 1.830, p > 0.05, df = 32) between the pulse of the animals in the apneic and the post-apneic phase (n = 24, *mean* = 63 beats/min, range 38 – 94 beats/min, SD = 13.8). The pulse of 8 animals was measured both during apnea and during the recovery phase. Also in this case there was no significant difference between pulse during the two periods (*t*-test, t = 1.233, p = 0.257, df = 7).

Rectal temperature during the apneic period measured 36.7°C (n = 6, range 36.2 – 37.5°C, SD = 0.51). During the same period peripheral temperature measured 35.2°C (n = 6, range 34.1 – 36.4°C, SD = 0.88). In the recovery phase internal temperature was still 36.7°C (n = 15, range 35.3 – 38.4°C, SD = 0.82), whereas peripheral temperature was 30.1°C (n = 13, range 13.9 – 36.1°C, SD = 6.11). For 4 animals internal temperature was recorded both during apnea and the recovery period, but no significant difference was found between the two (paired *t*-test, *t* = 0.960, p = 0.408, df = 3). Likewise, peripheral temperature measured at the hind flipper showed no significant difference between the same two periods (paired *t*-test, *t* = 1.143, p = 0.34, df = 3).

Of the 41 animals immobilized in this group, full immobilization was achieved in 32 cases, failure to restrain the animal occurred in 5 occasions and 4 animals died. Two of the casualties (see "Mortality") occurred during or immediately after handling.

Blood pH-values were measured in five animals between 20 min and three hours from induction. Values ranged between 6.8 and 7.2 (Figure 8).

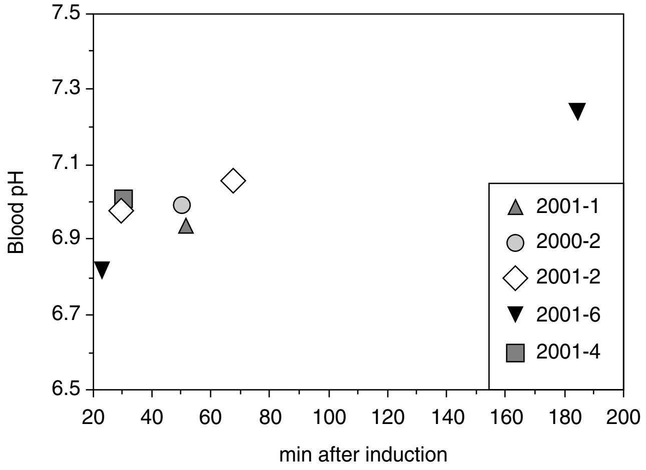

Figure 8 pH-values in blood of five Atlantic walrus (*Odobenus rosmarus rosmarus*) (Greenland 2001) during apnea and the post-apneic period following immobilization with etorphine.

Multiple immobilizations (group B)

This group was administered the agonist dose under the same conditions as for group ***A***. The estimated total body mass of the animals in group ***B*** ranged from 1050 to 1600 kg (Table 4). A total of between 4 and 9.8 mg etorphine was administered per individual walrus at an estimated mean dose of 6.4 µg/kg (Table 4).

Apnea was induced in all animals 4.1 min (Table 4) after injection of etorphine and apnea lasted 14.6 min (Table 4). A first dose of 11.3 mg (n = 35, range 3.0 - 21.0 mg, SD = 3.3) of the antagonist diprenorphine was administered at a mean of 7.8 min after darting (Table 4). The total dose of diprenorphine delivered to the animals that were successfully immobilized ranged from 7.7 to 32.2 µg/kg (Table 4).

The analysis of the effect of repeated anesthesia on the induction time did not show any significant correlation between the different immobilizations of the same individual. Neither induction time nor apnea duration varied significantly ($p > 0.05$) with subsequent immobilizations irrespective of the relative dose of the agonist or antagonist or the time incurring between the administration of the two substances. This suggests that repeated immobilization of an animal did not have any noticeable effect on induction time (i.e. no habituation to etorphine).

One animal (***1989-3*,** Table 2) was immobilized with etorphine twice in 1989 and once in 1990 and then treated again once in 2000 and twice in 2001. This ca. 31 old animal died during the last immobilization after it apparently had regained consciousness and was breathing autonomously.

In 2000 and 2001, a subsample of the animals that were captured and immobilized several times were included in a study of body water content, body water turnover and energy metabolism involving the use of stable isotopes (deuterium oxide dilution and doubly-labeled water) (Acquarone and Born *this thesis*; Acquarone et al. *this thesis*). It was essential for the study that the animals were kept sedated on the beach for repeated sampling of blood through the catheter during the time required for the equilibration of the injectates with the body water pool. In 32 cases it was possible to handle the animals taking advantage of their drowsiness (up to 2 hr) while they were recuperating from the etorphine / diprenorphine immobilization. In 6 cases it was necessary to administer medetomidine (reversed with antisedan) to prolong the period in which they were tractable (Griffiths et al. *this thesis*). In the immobilizations were the animals were sufficiently lethargic as a side-effect of the immobilization with etorphine, blood could be sampled up to 6.5 hr after darting with etorphine.

Mortality

The overall mortality rate was 7% (5 dead of 69). The five animals died during somewhat different circumstances: In 1989, one walrus had resumed controlled and regular breath but was found dead 230 min after having regained consciousness (first breath). In 2001, two animals had apparently regained breath after injection of the antagonist. However, their breath never became deep and regular and instead it became increasingly shallow until it stopped. Walrus ***1989-3*** (Table 2) had been immobilized several times between 1989 and 2001. Another animal became apneic after injection of etorphine and never breathed again. A fifth animal resumed regular breath and went into the water after having been treated. It was found dead, 8 days after immobilization, stranded in the tidal zone about 9 km from the haul-out.

Mortality was tested against etorphine dose, diprenorphine dose and timing of injection of the antagonist by bootstrap analysis, but there was no significant result for any of the factors ($p > 0.05$).

Another side effect experienced was inability to fully immobilize an animal. In 6 cases were the walruses not sufficiently immobilized to allow for treatment. In one case in 1989 the animal (***1989-3***, Table 2) never became fully immobilized whereas in the in five other failed immobilizations the subject woke up during the initial phases of the treatment. These latter animals escaped into the water where they were apparently able to swim and dive in a coordinated fashion.

The rates of mortality and failure to fully immobilize the animals apparently differed between the researchers in charge of the drugging (Table 5). However, these differences were not statistically significant (rate of mortality: $chi^2 = 6.82$; $p = 0.08$; $df = 3$; failure to fully immobilize an animal plus mortality: $chi^2 = 7.44$; $p = 0.06$; $df = 3$).

Table 5 Rates of mortality and failure to fully immobilize walruses in this study in relation to individual researchers in charge of the drugging. The name of the researchers has been kept confidential.

Researcher	Total immobilizations	Succeeded	No. failed (%)	No. died (%)	Year
1	17	15	1 (5.8)	1 (5.8)	1989, 1990, 1994
2	9	9	0 (0)	0 (0)	1991, 1992
3	22	22	2 (9.1)	0 (0)	2000
4	21	14	3 (14.3)	4 (7.3)	2001
All	69	60	4 (8.7)	5 (7.3)	1989-2001

In this study immobilization were attempted 69 times. It was possible to obtain full immobilization 58 times (84%), in six cases (9%) immobilization failed and the animals were never completely restrained, while five individuals (7%) died from the treatment for no apparent reason. Similarly Hills (1992) effectuated 59 attempts resulting in eight (14%) incomplete immobilizations and five (9%) casualties in Pacific walruses. Griffiths et al. (1993) experienced 2 partial immobilizations (5%) and one casualty (3%) during 38 immobilizations of walruses in Svalbard. During six immobilizations at Svalbard in the subsequent years, two more casualties were experienced (Griffiths unpublished). Overall mortality was 7% at Svalbard too. Mortality rates experienced in these studies do not differ significantly (chi^2-test, $p > 0.05$). Hence it can be concluded that etorphine reversed with diprenorphine may result in an overall mortality of 8% (13 died of 172 handled) in walruses.

Discussion

The mean induction time of 5.0 min reported in this study for etorphine doses between 2.4 and 12.6 µg/kg does not differ statistically ($t = 1.429$; $p > 0.05$; $df = 71$) from the average induction time of 5.7 minutes, reported by Griffiths *et al.* (1993), where 3.3 to 9.6 µg/kg etorphine was used on Svalbard on Atlantic walruses, which were of similar size to those in Greenland ($t = 1.567$, $p > 0.05$, $df = 67$).

In male Pacific walruses given etorphine the mean induction time reported was 15.4 min ($n = 25$, range 5.5 - 35.8 min, $SD = 8.1$) after administration of 7.3 µg/kg of the drug ($n = 21$, range 4.0 - 2.7 µg/kg, $SD = 0.39$) (Hills 1992). There was though a significant difference in body size between the animals in the present study and the ones reported in Hills (1992) (*t*-test, $t = 4.951$, $p < 0.001$, $df = 53$), the relative dose of etorphine administered was significantly lower (*t*-test, $t = 2.453$, $p < 0.05$, $df = 52$). When analyzing the data in Hills (1992), similarly to this study, it was not possible to find any correlation between dose of etorphine and induction time (linear regression analysis of dose – log-induction time; $p = 0.42$, $R^2 = 0.13$, $n = 51$). However, mean induction time in Pacific walrus was significantly longer than found here (*t*-test, $t = 7.532$, $p < 0.001$, $df = 61$). The surprisingly long induction time experienced in Hills' study may be ascribed to the shorter needles (6 – 8 cm) used for darting larger animals (some of the agonist may have been deposited in the skin and blubber) or to differences in the definition of "induction time". In both studies induction time is defined as the "number of minutes from darting until the animal is safe to approach", however the parameter "safe to approach" is subjective and depends on the experience of the operator.

Etorphine depresses respiration by raising the threshold of the reflex of inspiration and by affecting stretch receptors in the lungs (Kock et al. 1987). Generally apnea is induced at markedly lower levels of anesthesia in marine mammals than in terrestrial mammals (Hammond and Elsner 1977). The drug-induced muscle tonus and apnea observed in this study have also been reported when etorphine was used for immobilization of phocidae (Haigh and Stewart 1979; Parry et al. 1981). The duration of apnea in the present study was not significantly longer than in Pacific walruses (*mean* = 11.8 min, range 5.5 - 24.0 min, SD = 4.8, n = 22) (Hills 1992) (*t*-test, t = 1.407, $p > 0.05$, df = 56) and Atlantic walruses on Svalbard (*mean* = 11.9 min, range 5.6 – 20.8 min, SD = 3.8, n = 35) (Griffiths et al. 1993) (*t*-test, t = 1.778, $p > 0.05$, df = 69).

Aerobic dive limit in Atlantic walruses has been estimated at between 9.8 and 10.5 min for 1100 to1500 kg animals, respectively (Wiig et al. 1996; Nowicki et al. 1997). Observations of diving walruses in the wild indicate that this limit is rarely exceeded (Wiig et al. 1996; Nowicki et al. 1997). However, syntheses on body mass and diving capacity in pinnipeds (Boyd and Croxall 1996; Schreer and Kovacs 1997) indicate that maximum aerobic dive duration in a 1500 kg walrus may be close to 25 min which has been confirmed by observations in the wild (Gjertz et al. 2001). In the present study, in seven cases the animals survived apneas of over 20 min with a maximum of 35 min. The markedly increased respiration rate during the recovery phase observed in all immobilizations indicates that the animals may be compensating for oxygen deficiency built up during narcosis. Furthermore Griffiths *et al.* (1993) noted that walruses immobilized with etorphine remained lethargic during the recovery phase and suggested that the prolonged apnea produced metabolic acidosis. This supposition is confirmed by the measures of blood pH reported here. The generally smooth recovery and apparently unaffected *long-term* post-handling behavior observed both in previous (Born and Knutsen 1992b; Born and Knutsen 1997) and in the present study indicates a large tolerance towards apnea, hence low blood pH, in walruses during immobilization and suggests that respiratory and circulatory mechanisms serving long dives are maintained during anesthesia with etorphine.

Etorphine induces alterations in the number and distribution of μ-opioid receptors in the rat cerebral cortex if administered alone, but no apparent change was visible if etorphine administration was accompanied by the antagonist naloxone (Melone et al. 2000). With this in mind changes in the number and distribution of opioid receptors may accompany variations in the animals' tolerance to the drug. However, the analysis of repeated immobilizations of 10 individuals, both within and across the years, did not indicate any change in the effect of the drugs on the subjects. The fact that the animals in this study were subject to several immobilization during a relative short time frame indicates a substantial tolerance to etorphine and perhaps more noteworthy to one of its side-effect: prolonged apnea.

Accurate monitoring of physiological parameters, with particular attention to internal temperature, pulse, blood pH and glycemy, during immobilization is here advocated to acquire a deeper understanding of immobilization by this and other techniques.

A pulse of about 64 beats/minute during apnea and early recovery was observed. During the study at Svalbard (Griffiths et al. 1993) resting pulse ranged between 52 and 66 whereas during deep narcosis the pulse was between 22 and 48 beats per min. Griffiths *et al.* (1993) stated, without presenting the details, that heart rates remained steady, but varied somewhat with the dose of drug received and that animals receiving a high etorphine dose showed lowest pulse rates.

Etorphine is known to interfere with thermoregulation, causing hyperthermia or hypothermia dependent of ambient conditions and activity levels (Alford et al. 1974). However, the internal temperatures measured here were within the normal range reported for pinnipeds (Sweeney 1974; Whittow 1987) indicating that thermoregulation was not severely affected during immobilization. Also in other studies where walruses were immobilized with etorphine or carfentanil there have been no apparent thermoregulatory problems. Reported body temperatures in other studies ranged between 34.2 and 37.9°C (Hills 1992; Griffiths et al. 1993; Lanthier et al. 1999). The fall in peripheral body temperature reported in this study from 35°C to 28°C indicates that peripheral circulation control is not impaired by etorphine.

The blood pH-value measured during apnea for one animal (***2001-6***, Figure 8) was 6.8 which increased to 7.2 two hours after induction (corresponding to 2.7 hr of active ventilation). A second animal (***2001-2***, Figure 8) presented a blood pH of 6.9 at the end of apnea, which raised to 7.1 half an hour later. The low values of blood pH at the end of the apneic period are probably to be ascribed to the hypercapnia and reflects immobilization-induced stress. However, the blood pH became normal relatively shortly after regain of respiratory function.

The dosage of etorphine varies widely for all walrus studies mainly due to differences in animal TBM. The latter is a difficult parameter to assess at a distance in a field situation. However, after having experienced a case of under-dosage (4 mg corresponding to 2.4 µg/kg) in 1989, a total injection of between 6 and 10 mg etorphine irrespective of the size of the animals was routinely used. In most cases this resulted in successful immobilization of the animal. This confirms that it is advisable to administer the maximum dose of etorphine, rather than the minimum effective dosage, and then reverse promptly with an appropriate dose of antagonist (Alford et al. 1974; Booth 1988), because underdosing may cause severe physiological and behavioral reactions (Alford et al. 1974) as hyperexcitability, hyperventilation and subsequent alkalosis leading to death at a later time in the recovery phase.

Renarcotization has been suggested to explain the death of walruses immobilized with carfentanil and subsequently antagonized with naloxone (Hills 1992). In other mammals, renarcotization has been observed from 2 to 72 hr post immobilization (Haigh et al. 1983; Jessup et al. 1985; Seal et al. 1985; Jacobson et al. 1988; Allen 1990). The present study suggests that renarcotization could serve as an explanation for cause of death in the three cases where animals had regained breath and apparently had come out of the immobilization. In those cases sufficient amounts of diprenorphine were given. However, despite that this was routinely injected in more than one site to secure absorption, some of it may have been deposited in blubber.

The researchers who were in charge of the immobilizations prior to 2001 all had several seasons of experience with using this drug combination for immobilizing walrus. This is in contrast with the situation in 2001 when a relative high mortality and failure-to-immobilize rate was experienced. Familiarity with the effects of etorphine and diprenorphine in walrus and with walrus anatomy is in our opinion crucial. The secure administration of the antagonist into muscle or other metabolically active tissues is important to prevent suffocation. On the other hand injecting the antagonist directly into a vein or a blood vessel sinus may result in the animals waking up prematurely.

In this study both short term monitoring by visual observation and long term studies by satellite telemetry showed no sign of adverse effects of the immobilization treatment. However, the rates of mortality and of animals that woke up prematurely were relatively high. Furthermore the induction of prolonged apnea with associated acidosis is an undesirable side effect of this immobilization technique. It is here recommended that etorphine reversed with diprenorphine for immobilization of walrus be used only for short term handling, and that experiments in search for a more suitable agent for long-term immobilization be effectuated.

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Paper II: Prolonged chemical restraint of walrus (*Odobenus rosmarus*) with etorphine/diprenorphine supplemented with medetomidine/atipamezole.

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Abstract

Physiological studies involving the use of isotopic water created the need to hold free-ranging walruses (*Odobenus rosmarus*) restrained for several hours. In August 2000, six male walrus (total body mass: 1050-1550 kg) were immobilized in E Greenland by remote delivery of 8-9.8 mg of etorphine and subsequently restrained for up to 6.75 hr by administration of medetomidine. The effects of etorphine were reversed with 10-24 mg diprenorphine. After termination of the etorphine-induced apnea, lasting an average of 15.8 min (SD = 9.7, range = 9.5 - 35.2 min, n = 6), the animals were initially given 10-20 mg medetomidine intramuscularly. The initial dose was further augmented by 5 mg at intervals of 5 min. In two cases, when medetomidine was administered though a catheter inserted in the extradural vein, the animal became instantly apneic and regained respiratory function only after intravenous injection of the prescribed dose of the antagonist atipamezole and of the respiratory stimulant doxapram. After several hours of immobilization rectal temperature began to increase and it is conceivable that this is the factor that will ultimately limit the duration of immobilization. The animals became conscious and fully mobile shortly after an intravenous injection of a dose of atipamezole appoximately twice the mass of that of medetomidine followed by 400 mg of doxapram. It is concluded that medetomidine appears to be a suitable drug for chemical restrain of walruses for time-consuming procedures following initial immobilization by etorphine/diprenorphine. With animals of total body mass around 1,000-1,500 kg, the drug should be given intramuscularly in 10-20 mg increments (total mass 10-60 mg) until the breathing rate falls to approximately 1 min^{-1}. At this level, breathing is maintained and animals do not respond to touch or injection.

Keywords: walrus, immobilization, etorphine, M-99, Greenland, msedetomidine, marine mammal, doxapram, atipamezole.

Introduction

A variety of drugs or drug combinations has to date been used to immobilize walruses (*Odobenus rosmarus*) in the field, including Phencyclidine/Acepromazine (DeMaster et al. 1981), Tiletamine and Zolazepam (Stirling and Sjare 1988; Hills 1992; Griffiths et al. 1993), etorphine (Hills 1992; Born and Knutsen 1992; Griffiths et al. 1993; Acquarone et al. *this thesis*) and Carfentanil (Hills 1992; Lanthier et al. 1999). None have proven ideal for prolonged immobilization. Only Lydersen et al (1992) have tried a prolonged immobilization of walrus. The animal was initially immobilized by intramuscular (i.m.) administration of 5 mg (5.2 µg/kg) etorphine HCl. The effect of the etorphine was reversed, as prescribed, by its antagonist diprenorphine HCl. To keep control over the animal, a combined dose of 80 mg medetomidine HCl and 1000 mg ketamine HCl was then administered, before complete recovery, and provided restraint for further 5 hr (Lydersen et al. 1992).

An investigation of body water dynamics and energy expenditure in walruses in Greenland using isotope-labelled water (Acquarone and Born *this thesis*; Acquarone et al. *this thesis*) created the need to regularly immobilize and restrain the subjects for longer than typical to allow the injected isotopes to mix with the body water pool.

Materials and Methods

The fieldwork was conducted at a haulout at Lille Snenæs (Dove Bay, Greenland; 76°52.7´N, 19 37.9´W) during August 2000. Six adult male walruses (1050-1550 kg) were immobilized by remote administration of a dart containing 8-9.8 mg etorphine (“M99”, 9.8 mg/ml, Vericore Ltd., Dundee, Scotland) as described in Griffiths et al. (1993). Etorphine was antagonized by i.m. injections of 6-15 mg of diprenorphine (“M5050”, 12mg/ml, Vericore Ltd., Dundee, Scotland). Three of the animals were immobilized for the first time (animals 1-3: Table 6), one had been immobilized previously twice in 1989 and once in 1990 (animal 4: Table 6), the last two (animals 5, 6: Table 6) had been immobilized previously three times and twice respectively during the same month.

The etorphine/diprenorphine protocol used for immobilization of all walruses is described in detail in Acquarone et al. (*this thesis*). In this paper we only present information on a sub-set of six walruses that were treated with medetomidine/atipamezole after they had received etorphine and diprenorphine.

Some 30-40 min after the resumption of breathing, when the respiratory rate had fallen to around 8 min^{-1} and was not longer forced, six walruses were given an intramuscular injection of 10-20 mg medetomidine (“Domitor Forte”, 10mg/ml, Orion Pharma, Turku, Finland) (in the first animal only, a mixture of medetomidine and ketamine) every 5 min until it again was tractable and would allow the placement of a catheter (Becton-Dickinson Secalon-T, 2.0mm x 160mm, Medisinsk Utstyr AS, Oslo, Norway) into the epidural vertebral venous sinus for blood sampling. In two cases, medetomidine was at some point given intravenously (i.v.), this is not recommended (see Results and Discussion). A 25 cm-long luer-lock extension (Becton-Dickinson Connecta, Medisinsk Utstyr AS, Oslo, Norway) was attached to the indwelling catheter to facilitate blood sampling with minimum disturbance to the animal. The catheter and extension were flushed with heparinized saline following each blood sampling. Body temperature was measured using an electronic thermometer (DM852, Ellab, Copenhagen, Denmark) with a probe inserted approximately 20 cm in the rectum. Heart rate was monitored with an electronic pulse meter (Exersentry, Respironics Instruments, Inc., Monroeville, Pennsylvania 15146, U.S.A.) equipped with four sensors connected to 6 cm long needles inserted, as far as possible from each other, through the skin. The animals’ head was covered with a towel to shade the eyes from direct sun light and prevent visual stimuli during handling.

If an animal showed signs of arousal (head lift, body movement), it was given additional medetomidine i.m.or i.v. At the conclusion of the sampling period the walrus was given the α_2 antagonist atipamezole ("Antisedan", 5 mg/ml, Orion Pharma, Turku, Finland) at a rate of approximately twice the mass of medetomidine administered. The dose was given either half intravenously (i.v.) and half i.m., or wholly i.m.

If an animal went into apnea after the administration of medetomidine, it was given i.v. atipamezole at a rate of twice the mass of medetomidine injected, followed by 400mg of the respiratory stimulant doxapram ("Dopram", Wyeth Lederle, USA) also i.v.

In the case walrus 5 and 6, heparinized plasma was collected for measurement of plasma concentration of medetomidine by Orion Pharma, Turku, Finland.

Total body mass (TBM) was estimated from measurements of standard body length and axillary girth using the equations in Born et al. (2003).

All records of time given in the present paper are time in relation to initial impact of the dart containing etorphine.

Results

Weather conditions were fine during the immobilizations: 3-7°C; wind force 1-4 m sec^{-1}.

Six walruses received i.m. medetomidine following reversal of the etorphine with diprenorphine (Table 6). Two of these six animals were at some stage given medetomidine i.v., but in both cases this led to immediate apnea that could only be reversed by the antagonist atipamezole (discussed below). The first of the six animals received a combination of ketamine and medetomidine (see below, Animal 1). It stopped breathing too, and started again only after i.v. injection of atipamezole and doxapram. All subsequent animals received only medetomidine. The duration of immobilization ranged from 2.36 hr to 6.75 hr, and in all cases except one where the animal suddenly sat up and entered the sea without receiving the antagonist, arousal was induced by injection of atipamezole.

Table 6 Data on the use of etorphine/diprenorphine for the immobilization of six walruses that were further kept restrained by use of medetomidine/atipamezole directly after regaining full respiratory function. (*) = Minutes from darting with etorphine.

Animal no.	Day (Aug 2000)	TBM (kg)	Etorphine Amount (mg)	Apnea Start*	Apnea Duration (min)	Diprenorphine 1st inj.*	Diprenorphine Amount 1st inj. (mg)	Diprenorphine Amount total (mg)
1	5	1050	9.8	2.0	9.5	4.7	14.4	17.4
2	9	1090	9.8	1.9	10.3	5.3	12.0	12.0
3	15	1300	9.8	3.5	11.8	8.5	10.0	10.0
4	18	1550	9.8	3.8	35.2	8.3	12.0	12.0
5	19	1280	8.0	3.7	14.7	7.6	12.0	24.0
6	22	1250	8.0	4.6	13.5	7.3	12.0	12.0

Since the immobilizing routine and behavior of the animals varied, a brief description of each case will be given.

Walrus 1 (TBM 1050 kg) received 9.8 mg etorphine that was reversed with 14.4 mg diprenorphine (Table 6). Drug induced apnea lasted a total of 9.5 min and the animals was breathing in a controlled and regular manner at 17 min post darting. This animal was given 24 mg medetomidine and 260 mg ketamine i.m. at 1.28 hr post-darting and at 1.33 hr ceased breathing. At 1.42 hr it was given 70 mg atipamezole with 400 mg doxapram i.v. and resumed breathing 2 min later. It remained totally immobile for the next 25 min, when it stopped breathing again. This time, however, it could be aroused enough for head-lifting and breathing by physical stimulation of the muzzle, after which it would again lower its head and become apneic. It was aroused in this way every two minutes to breathe until 2.68 hr, when it was given a further 50 mg atipamezole i.m. At 2.72 hr it resumed spontaneous breathing, but otherwise lay immobile. At 2.97 hr a blood sampling was attempted, but this aroused the animal totally and stimulated it to enter the sea. It was recaptured five days later, from observations made before the new immobilization it behaved normally. Later in the season it was observed once again by the haulout.

Walrus 2 (TBM 1090 kg) was immobilized with 9.8 mg etorphine that was reversed with 12.0 mg diprenorphine. Apnea lasted 10.3 min and the breath became regular at 14 min post darting (Table 6). This walrus received 10 mg medetomidine i.m. at 35 min, which had no observable effect on breathing, and a further 5 mg i.m. at 45 min, which resulted in the breathing becoming more shallow, interspersed with occasional deep breaths. Immobilization was sufficient to allow the walrus to be rolled onto a scale and weighed. By 2.37 hr it had begun to move its flippers and body and was given 5 mg medetomidine i.v. It stopped breathing almost immediately, however, and at 2.43 hr was given 40 mg atipamezole and 500 mg doxapram i.v. At 2.50 hr it sat up, with apparent full reversal of all immobilization, entered the sea and swam strongly away. This animal was recaptured both 3 and 13 days later.

Walrus 3 (TBM 1300 kg) received 9.8 mg etorphine and 10.0 mg diprenorphine. Duration of Drug induced apnea was 11.8 min and the animal had regular breath at 21 min post darting (Table 6). Walrus 3 was given 10 mg medetomidine at 39 min (with no effect) and a further 5 mg at 44 min, after which the breathing rate fell from 12 to around 4 min^{-1}. The walrus remained immobile for the next 2.5 hr, after which it began to rock its torso and move its head with breathing. At 3.53 hr and again at 3.62 hr it received 5 mg of medetomidine i.m. after which it again lay still with regular breathing. At 6.67 hr it was given 30 mg atipamezole i.m., and four minutes later it sat up and walked into the sea, apparently fully awake. Walrus 3 was recaptured three days later and also three times the following year.

Walrus 4 (TBM 1550 kg) received 9.8 mg etorphine and 12.0 mg diprenorphine. Drug induced apnea lasted 35.2 min and regular breath was resumed 44 min post darting (Table 6). This individual received 12 mg medetomidine i.m. at 1.00 hr and a further 3 mg i.m. at 1.08 hr. Thereafter it lay immobile with regular breathing until about 1.50 hr, when it began to move its torso and rear flippers intermittently. It did not react, however, to insertion of a needle into the epidural space for blood sampling and was not given additional medetomidine. At 2.88 hr it sat up abruptly and entered the sea, apparently totally recovered and without the use of atipamezole. This animal had been already immobilized by etorphine darting both in 1989 and 1990 and was also immobilized twice the year following the events in this paper.

Walrus 5 (TBM 1280 kg) received 8.0 mg etorphine and 24.0 mg diprenorphine. Drug induced apnea lasted 14.7 min and at 18.5 min post darting the walrus had resumed regular breath (Table 6). Walrus 5 was received 20 mg medetomidine i.m. at 30 min. At 45 min it showed some body-rocking, and reacted to the insertion of a rectal thermometer and to tapping on a tusk, and was at 1.06 hr given a further 10 mg medetomidine i.m. At 1.83 hr it was given a 5 mg medetomidine i.v. and stopped breathing almost immediately. Several minutes later breathing had not recommenced and there was no response to the i.v. injection of 500 mg doxapram. At 1.90 hr it was given 25 mg atipamezole i.v. Shortly after it again began to breathe at a rate of about 1 min^{-1}, but continued to lie immobile without response to tactile stimulation. After 15-20 min this respiratory pattern became Cheyne-Stokes-like, with 3-5 deep breaths every couple of minutes.

Approaching 5 hr immobility, the breathing pattern reverted to a regular one, about 1 min^{-1}. At 5.48 hr the walrus was given 50 mg atipamezole, half i.m. and half i.v., and at 5.53 hr it sat up and entered the sea of its own accord, apparently normal. Even though this animal was not seen at haulout later this season, its movements have been followed by satellite tacking for more than 100 days after this immobilization.

Walrus 6 (TBM 1250 kg) received 8.0 mg etorphine and 12.0 mg diprenorphine. Drug induced apnea lasted 13.5 min and the animal had regained regular breath at 21 min post darting. This animal was given 20 mg medetomidine i.m. at 40 min, 45 min and 55 min, a total of 60 mg. After the third dose its breathing rate fell from 6 min^{-1} to 1-2 min^{-1}. As with walrus 5, its breathing changed to a Cheyne-Stokes-like pattern, with several good breaths every 1.5-2.5 min (Figure 9). The heart rate altered cyclically with the breathing, from typically around 30-45 min^{-1} between breaths to 65-70 min^{-1} just prior to and during breathing.

At 3.42 hr the animal showed signs of arousal, with some body-rocking and flipper movement, and shorter intervals between breathing. At 4.23 hr and 4.32 hr it lifted its head and moved a little forwards, but otherwise continued to lie quietly. At 4.78 hr it was given 100 mg atipamezole i.m., and at 4.88 hr it sat up and entered the sea without apparent sedation. Walrus 6 was a frequent occupant of the haulout the following year when it was immobilized further 3 times.

Plasma medetomidine concentration was measured in animals 5 and 6. In both animals, concentration was initially low, then increased to variable levels during the following hours (Table 7). There was no apparent consistent trend in plasma medetomidine concentration with time, but it is worth noting that the concentration did not taper with time and was still high at the time of reversal of the drug.

Table 7 Medetomidine concentrations in blood plasma of two adult male walrus in relation to time during immobilization involving the initial use of etorphine/diprenorphine.

Time after etorphine darting (h)	Plasma medetomidine concentration (ng/ml)	
	Walrus 5	Walrus 6
0.5 (before medet.)	Undetectable	
1.5		2.81
2.0		19.7
2.5	9.24	18.6
3.0	9.82	13.7
3.5	5.11	9.42
4.0	12.1	9.66
4.5	11.5	15.0
5.0	6.33	
5.5	5.54	

Rectal temperature was measured in animals 3, 4, 5 and 6 (Figure 10). Rectal temperature shortly after initial immobilization with etorphine ranged 35.8-37.0°C, and remained below 38.0°C for some 2-3 hr. Thereafter there was a tendency for the rectal temperature to increase slowly to 38°C and above, although it never reached a point where it caused concern.

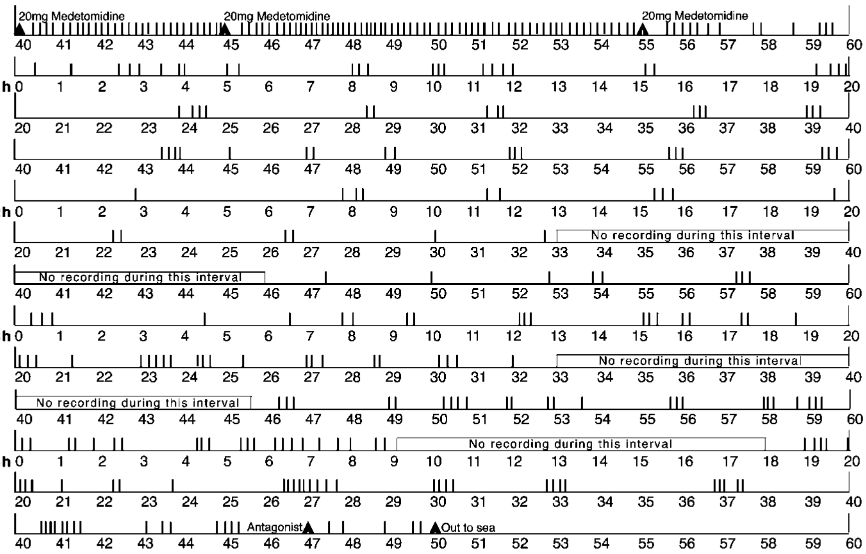


Figure 9 Breathing rate of an adult male walrus (no. 6) during immobilization with medetomidine following an initial immobilization with etorphine/diprenorphine. Time is given relative to darting with etorphine (i.e. the first dose of medetomidine was injected 40 min after darting). The breathing rate was greatly reduced after the third medetomidine injection, and after a further 0.5 hr was transformed into a more intermittent pattern with several breaths at intervals of several minutes.

Discussion

The choice of employing medetomidine for restraint after immobilization by etorphine was taken after considerations on employing medetomidine as the immobilizing agent instead of etorphine. However, initial trials in 1999 with medetomidine and Telazol® in two animals proved fatal in both cases (Griffiths unpublished) and indicated that this combination is not suitable for immobilization without extensive testing. Considering the small walrus populations on both Greenland and Spitsbergen, it was decided not to risk more casualties and to revert to the familiar immobilization drug, etorphine, as the first drug.

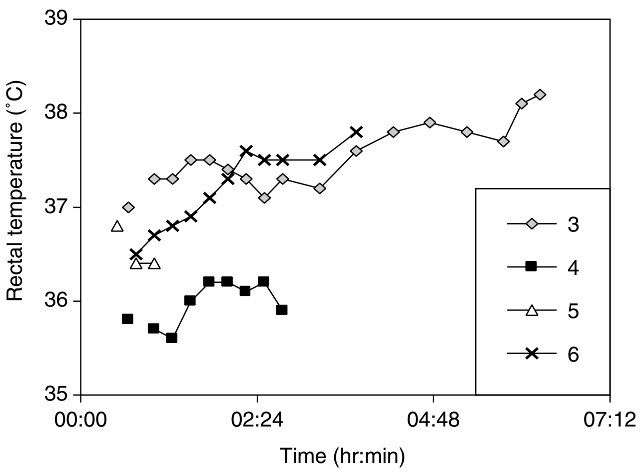
Medetomidine appeared to be very suitable as a second immobilising agent, following etorphine, when prolonged immobilization of walruses is necessary. When the animal appears to have recovered from the initial etorphine drugging, and is breathing regularly and unlabored at a rate of 6-8 min^{-1} (which for the animals in this study was at about 35-40 min from darting) it may be given medetomidine, using respiratory rate and pattern as an indicator of depth. medetomidine is to be given i.m. at 10-20 mg increments every five minutes until the respiratory rate falls to approximately 1 min^{-1}. The amount of medetomidine required to achieve this effect ranged in this trial between 15-60 mg. In the two animals immobilized the longest (5 and 6), the breathing pattern subsequently changed to an intermittent type, with bursts of several breaths interspersed with periods of apnea up to 4 min in duration.

The large variation in the dose of medetomidine needed to immobilize the walruses (15-60 mg) is probably related to the effects of the initial etorphine drugging. After reversal of etorphine with diprenorphine, some walruses will quickly become mobile and enter the sea of their own accord, reacting aggressively to human proximity. Others will recommence breathing and show head-lift, but appear unwilling to ambulate, do not respond to physical contact other than with head-lift, and may remain in the same position for over a day. This variation in recovery time may indicate residual central nervous system depression, which in turn may influence the amount of medetomidine needed to re-immobilize the animals.

There was also considerable variation in the duration of medetomidine immobilization before topping up was needed, from 2-3.5 hr. Signs of arousal from sedation included rocking of the torso in association with breathing, movement of the rear flippers, head movement and increase in respiratory rate to at least one breath per minute. Repeated dosage should also be administered i.m. and in small increments, with respiratory rate again being the key indicator of level of sedation.

In all cases intravenous administration of medetomidine, even a dose as small as 5 mg, led to immediate cessation of breathing, and the same thing happened in a previous attempt to immobilise a walrus with medetomidine (Lydersen et al. 1992). Only the use of the antagonist atipamezole could start breathing again. We conclude after several attempts to administer medetomidine i.v. that this route is absolutely contraindicated in the walrus, at least following the use of etorphine.

Figure 10 Rectal temperature in relation to time (min after darting with etorphine) in four adult male walruses that were immobilized with medetomidine.



For the four animals where data were available, rectal temperature remained nearly unaltered for a couple of hours of immobilization (Figure 10). No attempts were made to cool the animals or otherwise regulate body temperature during immobilization. There was a trend for temperature to increase with prolonged immobilization to around 38°C by the time the immobilization was reversed. Whether rectal temperature would have continued to increase or have leveled off is unknown. The fact that the rectal temperature increased in those three animals where it was monitored for more than two hours indicates that medetomidine may impair thermoregulation in walrus.

In summary, medetomidine appears to be a suitable drug for immobilization of walruses for time-consuming procedures following initial capture with etorphine and reversal with diprenorphine. With animals of weight around 1,000-1,500 kg, the drug should be given intramuscularly in 10-20 mg increments (total mass 10-60 mg) until the breathing rate falls to approximately 1 min^{-1}. At this level, breathing is maintained and animals to not respond to touch or injection. A rectal thermometer should be used, since after several hours of immobilization rectal temperature begins to increase and it is conceivable that this is the factor that will ultimately limit the duration of immobilization possible.

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Paper III: Body water and body composition of free-ranging Atlantic walruses (*Odobenus rosmarus rosmarus* L.) studied by isotope dilution

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Abstract

Deuterium oxide was used to measure isotope dilution space 11 times in seven free-living, adult, male Atlantic walruses (*Odobenus rosmarus rosmarus* L.) (930 - 1597 kg) in N.E. Greenland, August 2000-2001. Equilibration of the intravenously injected isotope was complete after 2-3 hours. The application of a general correction factor developed for all pinniped species allowed the estimation of total body water content to 56.8% (range: 36.6-73.3%) of total body mass (TBM). Water turnover averaged 44.5 g/kg * day. For one animal it was possible to estimate water influx to 4.8 g/kg * day and water efflux to 21.8 g/kg * day. The estimates of body fat and body protein averaged respectively 24.7% and 18.4% of TBM. Similarly, for a walrus with a TBM of 1300 kg body ash was calculated to 26 kg and body gross energy to 18300 MJ. The assessment of body fat by isotope dilution did not differ significantly from the estimates of blubber content obtained from the dissection of walruses in the wild. This work demonstrates the feasibility of using the hydrogen isotope dilution technique for estimating body composition of walruses.

Introduction

The subcutaneous adipose tissue layer (blubber) is often used to assess body condition of pinnipeds (Gales and Renouf 1994; Gales et al. 1994a) as it represents the main energy store for breeding and molting (Bryden 1968). Hence, individuals with better body condition have greater chances of reproductive success (Bowen et al. 1994; Arnbom et al. 1997; Pomeroy et al. 1999). Proper estimation of fat content, hence body condition, is relevant for the evaluation of the responses at an individual level to major changes in the carrying capacity of a population (Fay et al. 1989).

Direct chemical analysis of body composition of seals has been carried out only on five species (Reilly and Fedak 1990; Lydersen et al. 1992b; Oftedal et al. 1993; Gales et al. 1994a; Arnould et al. 1996; Oftedal et al. 1996), not including walrus. Walrus body composition has been investigated only by dissection and weighing of body components (Knutsen and Born 1994).

Blubber is the only body component measurable directly in field studies by non-destructive methods. To date the only direct, non-invasive, non-destructive technique available to measure the thickness of the blubber layer of live seals is ultrasonography (Slip et al. 1992; Webb et al. 1998). Alternatively, the amount of an animal's body fat can be indirectly estimated *in vivo* from measures of total body water (TBW) by hydrogen isotope dilution analysis (IDA) (Sheng and Huggins 1979; Nagy and Costa 1980; Costa 1987; Oftedal and Iverson 1987; Reilly and Fedak 1990; Slip et al. 1992; Arnould et al. 1996; Bowen and Iverson 1998; Beck et al. 2003) or by bioelectrical impedance analysis (BIA) (Gales et al. 1994b; Arnould 1995; Bowen et al. 1999).

The species of pinnipeds for which validation of isotope dilution studies exist to date are Antarctic fur seals (*Arctocephalus gazella*) (Arnould et al. 1996), Gray seals (*Halichoerus grypus*) (Reilly and Fedak 1990), Ringed seal (*Phoca hispida*) (Lydersen et al. 1992b), Hooded seal (*Cystophora cristata*) (Oftedal et al. 1993) and Harp seal (*Phoca groenlandica*) (Oftedal et al. 1996).

Bioelectrical impedance analysis has proven fast, inexpensive and reliable for humans (Lukaski et al. 1986; Kushner et al. 1990), bears (Farley and Robbins 1994), horses (Forro et al. 2000), pigs and sheep (Jenkins et al. 1988; Swantek et al. 1992). This technique is generally practical for field studies due to the light experimental setup. But though BIA seems to perform well for some species of pinnipeds such as harp, ringed (Gales et al. 1994b) and gray seals (Bowen et al. 1999), it appears to be a poor predictor for TBW in others such as female Antarctic fur seals (Arnould 1995) and in both female and male harbor seals (*Phoca vitulina*), though it performed better with suckling pups (Bowen et al. 1998) of the same species. Bowen *et al.* (1998) reported that in spite of sedation, the immobilized seal reacted to handling with muscular contractions that possibly induced great variability in the BIA measures. They stated that (a) chemical anesthesia actually improves the precision of the technique and (b) that instead of using them independently, BIA measurements can be "a valuable adjunct" to the more precise IDA techniques (Bowen et al. 1999).

Among the more precise methods, the hydrogen (tritium or deuterium) IDA technique has been widely used on several species of vertebrates in field situations where it can be utilized alone or coupled with other IDA techniques (e.g. ^{18}O) for the determination of energy expenditure (Nagy 1987; Speakman 1997; Nagy et al. 1999; Acquarone et al. *this thesis*), water flux (Depocas et al. 1971; Nagy and Costa 1980; Lydersen et al. 1992b; Boyd et al. 1993; Lea et al. 2002) food consumption (Costa 1988; Costa et al. 1989; Nagy et al. 1999) and milk transfer between mother and offspring (Lydersen et al. 1996; Carlini et al. 2000; Donohue et al. 2002). Several authors consider IDA to be a very precise technique for pinnipeds, provided sufficiently long immobilization time for isotope equilibration (up to 3 hours) (Bowen and Iverson 1998).

The hydrogen IDA technique has been chosen for this study because of its superior precision compared to other techniques to measure the hydrogen dilution space, and thus infer TBW and body fat, in free-living walruses. The body fat content thus derived has been compared with direct measures of walrus body composition from animals killed during the Inuit's traditional hunt in Greenland (Knutsen and Born 1994) and with one animal that was dissected *in situ* in NE Greenland in 2001 (this study).

The data presented in this work may form the basis for a deeper understanding of the physiology of this species and it provides essential data for future studies involving isotope dilution.

Materials and Methods

Study site

The study was carried out during August in 2000 and 2001 at Sandøen (74°15.7'N, 20°09.7'W) and at Lille Snenæs (76°52.7'N, 19°37.9'W) in North-East Greenland, which are the only two regularly-used, terrestrial haulouts for walruses in Greenland (Born et al. 1995).

Choice of animals

Fourteen individual walruses were chosen among the all-male groups using the haulout. Within the study period some animals were captured several times within the same year and between the years (Table 8) (Project approval of the Greenland Home Rule, file:28.40.10). Animal choice criteria were: sufficiently large tusk size for instrument attachment, quiet behavior for effective immobilization, relative placement farthest from the shoreline to minimize risk of escape at sea in the anesthesia induction phase and relative placement clear of the other hauled-out individuals for safer remote injection of the immobilizing agent (Born and Knutsen 1992a).

Immobilization

Before handling the animals were completely immobilized by remote injection of etorphine HCl when hauled-out on land, using the protocol described in Born and Knutsen (1992a), Griffiths *et al.* (1993) and Acquarone et al. (*this thesis*). When effectively immobilized the animals were approached and rolled supine after which their length and girth were measured (American Society of Mammalogists 1967) for estimation of total body mass (TBM) (Born et al. 2003), the instruments were attached to the tusks and venous access for blood sampling and isotopic enrichment was gained by catheterization of the epidural vein in the lumbar region. After completion of this handling, the animals were usually allowed to regain consciousness and to escape to sea at will. In some cases the animals were kept sedated for up to 7 hours (Griffiths et al. *this thesis*) to investigate the dynamics of isotopic equilibration. Upon recapture a similar immobilization procedure was effectuated followed by somatic measuring, blood sampling and instrument data retrieval.

Water metabolism

At initial capture the venous blood was sampled through the epidural catheter for determination of background isotope concentration. An intravenous dose of either concentrated (99.8%) deuterium oxide (98 to 148 ml) or of doubly labeled water (98 to 158 ml, containing 44% deuterium oxide-99.8%) or of a saline solution of deuterium oxide (629 ml, 5.3% and 996 ml, 4.7%) was subsequently administered. In all cases the source of deuterium oxide was spectroscopy grade heavy water (Merck 1.13366, E.Merck, D-6100 Darmstadt, Germany). In 7 cases it was possible to sample blood at regular intervals from the infusion of the isotope. This allowed the determination of isotope concentration in the blood for up to 6 hours from enrichment. The subjects returned to the beach and were re-immobilized after 3 to 19 days from isotopic enrichment, blood was sampled again either through an epidural vein catheter or in two cases by bleeding the hind flipper plexus. On one occasion it was possible to re-enrich the animal and subsequently to obtain a second series of blood samples. At all times whole blood was directly poured in 2-ml standard glass vials immediately after sampling and flame sealed.

Seawater background samples were also collected at both study locations and immediately flame sealed in 2-ml glass vials to investigate fluctuations in ambient isotope concentrations.

All samples were stored at ambient temperature (max 12.9°C, usually <5°C) while in the field and were subsequently kept refrigerated at 5°C prior to analysis.

Table 8 List of male Atlantic walruses included in this study of total body water (TBW) and water turnover rate (WTR) in NE Greenland in 2000 and 2001. Blood from all animals was sampled to estimate isotope background enrichment. The localities where the study was conducted were Lille Snenæs (LSN) and Sandøen (SND). The model of the instruments attached is shown (TDR = Time Depth Recorder, SLRT = Satellite Linked Radio Transmitter, Wildlife Computers, Redmond WA, USA).

Year	Animal ID	TBM (kg)	No. of Captures	Locality	TBW	WTR	TDR	SLRT
2000	B	1280	4	LSN	x	x	Mk7	SDR-T10
	C	1250	3	LSN	x	x		SDR-T10
	D	1050	2	LSN				
	E	1120	3	LSN	x	x	Mk7	SDR-T10
	F	1090	3	LSN	x	x	Mk7	
	G	1310	2	LSN	x	x	Mk7	SDR-T10
	H	1550	1	LSN				SDR-SSC3
	A	930	1	SND				
2001	A	1230	1	SND				SDR-T10
	I	880	1	SND				
	J	1410	1	SND				SPOT2
	K	1070	1	SND				SPOT2
	L	840	1	SND				
	M	1170	1	LSN				SPOT2
	H	1550	2	LSN	x	x	Mk7	SPOT2
	C	1250	3	LSN	x	x	Mk7	SPOT2
	G	1370	3	LSN	x	x	Mk7	SPOT2
	N	1600	2	LSN				SPOT2

Sample analysis

In preparation for mass spectroscopy, all blood samples were vacuum distilled into Pasteur pipettes (Speakman 1997) and the distillate was used for determination of ^{2}H concentration. $^{2}H_2$ gas was produced by reduction with excess $LiAlH_2$ as described in Ward et al.

(2000) The isotopic composition of the injectate was measured by diluting a weighed quantity of the injectate (0.1 - 0.2 ml) into a weighed quantity of tap water (60 ml). This mixture was then treated in exactly the same manner as the distillate from the blood samples. In each batch of samples for analysis, laboratory standards were included to account for day-to-day variation in the analyzer. All isotope enrichments were measured in δ-units in relation to an international standard reference gas and converted to absolute part per million (ppm) using the established ratios for reference materials.

Calculations of isotope dilution space and water turnover rate

Calculations of hydrogen isotope dilution space size were made according to the method described in Speakman (1997). In particular the values of dilution space and fractional turnover rate (K_d) were calculated using the iterative calculation method assuming steady state. TBW was estimated from the hydrogen isotope dilution space using a general regression equation developed to correct for the overestimation of TBW from desiccation [TBW = 0.003 + 0.968 * (H-dilution space)] (Bowen and Iverson 1998). The equations (3), (4) and (6) from Nagy and Costa (1980) were used for determination of water flux (WF).

Body composition

Body composition was calculated from estimates of TBW derived from the hydrogen dilution space. All walruses for which a TBW estimate was available were included in the calculations for body composition except walrus M (Table 8). This individual's estimated TBW content indicated an extremely lean animal when used in conjunction with the established allometric equations. For all other individuals total body fat (TBF) was calculated according to the general allometric equation for mammals [% TBF = 100 - (% TBW/0.732)] (Pace and Rathbun 1945). The values derived from these equations were compared to the similar results from the allometric equation for total body fat, total body protein (TBP), total body ash (TBA) and total body gross energy (TBGE) derived for gray seals [% TBF = 105.1 - 1.47 * (% TBW);% TBP = 0.42 * (% TBW) – 4.75; TBA (in kg) = 0.1 – 0.008 * (TBM in kg) + 0.05 * (TBW in kg); TBGE (MJ) = 40.8 * (TBM in kg) – 0.4] (Reilly and Fedak 1990). These estimates of % TBF were compared using two-tailed t-tests for data with unequal variances (Zar 1999) to the body composition of walrus estimated by dissection (Knutsen and Born 1994, and this study).

On 21 August 2001, an adult male (H) with a TBM of 1550 kg was dissected after it had died in connection with handling. Skin, blubber, muscle and all internal organs were weighed separately following the methods in Knutsen and Born (1994).

Satellite-linked radio transmitter and time depth recorder data analysis

Either an ARGOS System SDR-T10 or SDR-SSC3 Satellite-linked radio-transmitter (SLRT) with Time at Depth Histograms (6 animals) or a SPOT2 SLRT with time-at-temperature histograms (7 animals) and a Mk7 time depth recorder (TDR) with 500 m range in 2000 (4 animals) and 1000 m in 2001 (3 animals) (all instruments: Wildlife Computers, Redmond WA, USA) were attached to the tusks of the walruses using the method in Born and Knutsen (1992b) and Gjertz *et al*. (2001). Six animals were equipped with a SLRT only and one with a TDR only (Table 8).

In addition to providing data on location, the SDR-T10 and SDR-SSC3 transmitters were able to collect dive data to a depth of 250 or 500 m with a resolution of 1 or 2 m, respectively. Information on haul-out and at-sea time was collected by two of the SLRT in 2000 via ”timelines” (TIM) (Born et al. 2002). The satellite transmitters, their sampling protocols and data processing are described in Born *et al.* (2002) and Acquarone and Born (*this thesis*).

During August the walruses were immobilized and handled several times at the beach. It cannot be excluded that handling, and in particular the prolonged anesthesia, may have affected their natural behavior to an unknown extent. Immobilization can cause some post treatment drowsiness (Born and Knutsen 1992a; Griffiths et al. 1993). Because the study animals were equipped with SLRT’s we were able to compare their overall behavior during August when disturbance occurred with their behavior during September when there was no disturbance. For that purpose the information obtained via the SDR-T10 SLRT’s on TIM, time-at-depth, daily-average-dive-depth, and daily-max-dive-depth (DMDD) was used.

The TDR data were analyzed using the software provided by the manufacturer (Zero-Offset-Correction and Dive-Analysis). Periods when the walruses were hauled out on land or ice were identified from the instrument’s temperature profile and excluded from the analysis of dive activity. Minimum depth for a dive to be considered a foraging dive was assumed to 6 m. The time spent at sea or out of the water was determined from the temperature records of the TDR (only temperatures below 2.5°C were considered as originating from a submerged sensor). Number of dives per day, dive duration and surface times were also determined for each individual.

Correlation between Water Turnover Rate and Activity

Linear regression was employed to investigate correlation between activity levels and water turnover rate. The activity parameters chosen for comparison with water turnover rate during the monitoring period included “% time hauled-out”, “% time > 6 m” (*i.e.* likely foraging dives), “number of dives”, “average dive depth” and “average dive duration”.

Results

Background concentration of deuterium

In 2001 background deuterium concentration in seawater at Lille Snenæs averaged 148.2 ppm (SD = 8.1, range 131.2 - 168.6 ppm, n = 20). Background deuterium concentration in walrus blood averaged

152.9 ppm (SD = 6.5, range 138.5 - 165.0 ppm, n = 17). The walrus blood values did not show significant differences between the two sampling locations: Sandøen and Lille Snenæs. However, there were significant differences between the two sampling years ($t = -4.48$, $p < 0.05$, $df = 15$). The mean values for 2000 and 2001 were 148.4 ppm (SD = 4.4, range 138.5 - 152.4 ppm, n = 9) and 158.0 ppm (SD = 4.5, range 152.8 - 165.0 ppm, n = 8) respectively. In 2001, when deuterium concentration was measured simultaneously in sea water and walrus blood, background enrichment levels in the blood of the animals did not differ significantly from sea water enrichment ($t = 0.82$, $p > 0.05$, $df = 7$).

Isotope equilibration time and dilution space

The time required for complete equilibration of the deuterium bolus within the body water pool was estimated visually to 2-3 hr from the graphs of decline in isotope concentration for seven enrichments (Figure 11).

The mean TBW content was 56.8% of TBM (SD = 9.3, range 36.6 – 73.3%; n = 11) (Table 9). In 2000, the isotope dilution space size for animal C was measured twice, 19 days apart. An increase in TBW from 50% to 60% of TBM was seen between the first and the second measure. Three animals were captured both in 2000 and 2001. Between the two years, the estimates of TBW had decreased to 37% of TBM for animal C, while for animal G and H, TBW decreased from 63% to 55% and from 60% to 56% of TBM, respectively.

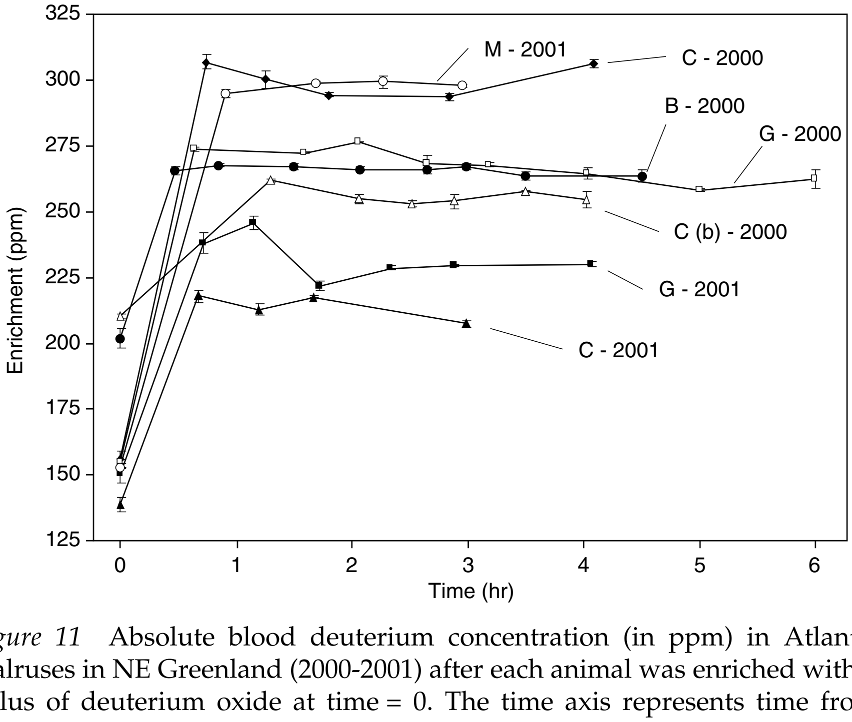

Figure 11 Absolute blood deuterium concentration (in ppm) in Atlantic walruses in NE Greenland (2000-2001) after each animal was enriched with a bolus of deuterium oxide at time = 0. The time axis represents time from enrichment. The concentration axis represents absolute concentration of deuterium. The enrichment year is shown after each animal ID. In 2000 animals B and C were enriched twice, for B only the second enrichment is shown, for C both enrichments are shown (C and C(b)). Animals C and G were enriched with deuterium both in 2000 and 2001. The bars indicate the SD of the measures.

Table 9 Measures of dilution space by deuterium dilution and estimated total body water (TBW) expressed in kg and % of body mass in Atlantic walruses in NE Greenland, 2000-2001. The estimate of TBW was derived from deuterium dilution according to the allometric equation for pinnipeds in Bowen and Iverson (1998).

ID	Date	Eq.time (h)	Water Dilution Space				TBW (kg)	TBW (% TBM)
			Mean (kg)	Min (kg)	Max (kg)	SD		
C	3-Aug-00	2.83	651	635	668	14	630	50.3
E	7-Aug-00	1.10	614	589	641	22	595	53.3
F	9-Aug-00	2.00	723	712	734	9	699	64.4
G	15-Aug-00	2.65	851	830	872	21	824	63.0
H	18-Aug-00	1.22	957	927	987	31	926	59.6
B	19-Aug-00	2.65	707	675	739	33	684	53.3
C	22-Aug-00	2.52	770	748	793	19	746	59.5
M	3-Aug-01	2.27	888	886	890	1	860	73.3
H	6-Aug-01	0.85	889	885	894	5	861	55.7
C	7-Aug-01	2.28	474	474	540	25	458	36.6
G	16-Aug-01	2.82	783	760	822	26	758	55.2

Water Turnover Rate and Water Flux

Mean water turnover rate (WTR) was 44.5 g/kg * day (SD = 29.6; range 14.0 – 96.2 g/kg * day; n = 8) (Table 10). For walrus C it was possible to measure TBW for twice within the same season which allowed to estimate water flux (WF) according to equations (4), (5) and (6) in Nagy and Costa (1980). There was no difference between the water efflux calculated for body water volume changing linearly or exponentially with time respectively with equation (4) and (5) and therefore only the results of equation (4) are presented.

Body Composition

Total body fat calculated from the equation of Pace and Rathbun (1945) averaged 24.7% of TBM (SD = 10.8, range 12.0 - 50.0%, n = 10). The corresponding value calculated from the equation for gray seals of Reilly and Fedak (1990) was 24.1% of TBM (SD = 11.6, range 10.4 - 51.3%, n = 10). There was no significant difference ($t = 0.12$, $p > 0.05$, df = 18) between the two estimates. Neither of the calculations of TBF (Pace and Rathbun 1945; Reilly and Fedak 1990) differed significantly from the mean value of 18% (SD = 4.3, range 11 - 26%, n = 15) for the measures of blubber content in relation to TBM for North West Greenland walruses of all ages and both sexes (Knutsen and Born 1994) (two tailed *t*-test - unequal variances, $t = 1.88$, $p > 0.05$, df = 10 for Pace and Rathbun (1945) and $t = 1.59$, $p > 0.05$, df = 10 for Reilly and Fedak (1990). However, there was a significant difference between these estimates of TBF and the mean value of 15% (SD = 2.8, range 11 - 19%, n = 7) reported in the same study for adult males only (two tailed *t*-test - unequal variances, $t = 2.72$, $p < 0.05$, df = 10 for

Pace and Rathbun (1945) and $t = 2.38$, $p < 0.05$, df = 10 for Reilly and Fedak (1990).

Table 10 Fractional turnover rate (K_d), half life ($T_{½}$), total body water (TBW), water turnover rate (WTR) for 6 different male Atlantic walruses and water efflux (WE) and water influx (WI) for one of these animals (walrus C) during a study in NE Greenland, 2000-2001. Water influx and water efflux were calculated according to (eq.4) and (eq.6) in Nagy and Costa (1980) respectively.

Year	ID	Measure Period (d)	Kd (/d)	T½ (d)	TBW (kg)	WTR (g/kg.day)	WI (eq.6) (g/kg.day)	WE (lin.eq.4) (g/kg.day)
2000	B	12.0	0.098	7.1	684	52.1		
	C	19.0	0.049	14.2	630	24.5	4.8	21.8
	E	11.0	0.180	3.8	595	96.2		
	F	13.1	0.119	5.8	699	76.7		
	G	2.8	0.022	31.3	824	14.0		
2001	C	8.7	0.057	12.2	458	20.9		
	G	4.9	0.042	16.7	758	22.9		
	H	14.9	0.088	7.9	861	48.9		

The dissection of animal H, produced a value for blubber content in relation with body mass of 21%. Some 15 days previously, and after an equilibration time of 0.85 hr its TBF was estimated to 23 and 24% by the equation of Pace and Rathbun (1945) and Reilly and Fedak (1990) respectively.

The animals in this study weighed on average 1300 kg (SD = 155, range 1086 – 1554 kg, n = 10). Total body protein averaged 18.4% (SD = 3.3, range 10.6 – 22.3%), TBA averaged 25.6 kg (SD = 6.0, range 13.0 – 34.0 kg) and TBGE averaged 18300 MJ (SD = 5044, range 10384 – 28887 MJ) (method from Reilly and Fedak 1990).

A comparison of disturbed and undisturbed behavior

First it was explored to what extent SLRT and TDR data on haul out behavior were similar during the period of deuterium oxide dilution experiments, as the two instruments data recording method differs considerably in resolution. For only two animals in 2000 (B and G: Table 8) simultaneous TIM data were available from both instruments in August. For B the total time hauled out during the experimental period was 30 and 20% recorded by the SLRT and the TDR, respectively. The corresponding values for G were 51 and 55%, respectively. This indicates that data from the two instruments reflected the haul-out activity reasonably similarly.

This conclusion may also be valid if single haulouts are compared. During the experimental period both instruments registered five full haulout events. The duration of these haulout periods recorded in both instruments were well correlated ($r = 0.97$, $Z = 3.06$, $p < 0.05$, $n = 5$).

Table 11 Time (%) at sea by month spent below 6 m depth during August and September 2000 by 5 adult male Atlantic walruses in NE Greenland (2000) calculated using SLRT data alone (animals C, H) and integrating time at depth (TAD) data from SLRT and time depth recorder (TDR) data (animals B, E, G). The dates in August and September and the number of hours monitored are showed. All animals except walrus H were administered deuterium oxide.

Month	ID	% time > 6 m	Dates	Hours monitored
Aug	B	37	3-27	51 . 6hr
	C	26	21-31	13 . 6hr
	E	8	11-28	23 . 6hr
	G	25	15-29	34 . 6hr
	H	53	18-31	30 . 6hr
Sep	B	68	13-30	26 . 6hr
	E	16	1-19	13 . 6hr
	H	66	1-30	91 . 6hr

Among the four animals enriched with deuterium oxide (B, C, E, G), that transmitted information during August 2000 on time spent at different depths (TAD), three spent between 25 and 37% of the "at sea" time below 6 m depth (i.e. they were possibly diving for food) (Table 11). However, animal E only spent ca. 8% of the "at sea" time below 6 m. The activity of B and E was also followed during September. Both animals used more time (B = 68%, E = 16%) below 6 m in September than in August (Table 11). In comparison, walrus H, which in 2000 was not enriched with deuterium oxide, spent 53 and 66% of its time below 6 m in August and September respectively (Table 11).

During August 2000, the percentage of dives below 6 m varied between 46 and 56% in the enriched animals, whereas it was 36% in the one animal that was not enriched that year (H) (Table 12). In September both of the enriched animals (B, E) dived comparatively more below 6 m than in August (93 and 64% of dives respectively) (Table 12). Animal (H), not enriched in 2000, also dived markedly more below 6 m in September (53%) than in August (36%) (Table 12).

In three walruses (B, E and G in 2000) it was possible to compare DMDD between deuterium enrichment and blood sampling with maximum depths recorded after the last blood sampling (Table 13). Only one of the animals presented a near significant difference ($p = 0.05$) between dive depths during the two periods (G). However, for this animal only two days with a record of maximum dive depth during the deuterium dilution measurement period were available.

These data indicates that in August both the enriched and the not-enriched animals spent less time below 6 m than in September and that in August they seem to dive more frequently to depths above 6 m. However, the lack of significant difference in mean daily maximum dive depth between the experimental period for the isotope dilution study and the following period, indicates that they were not influenced in their behavior by the experimental procedures.

Table 12 Percentage of dives at two depth intervals in August and September 2000 by 5 adult male Atlantic walruses in NE Greenland (2000) calculated using SLRT data alone (animals C, H) and integrating daily average dive (DAD) data from SLRT and time depth recorder (TDR) data (animals B, E, G). The dates in August and September and hours monitored are shown. All animals except walrus H were administered deuterium oxide.

		% of all dives				
Month	ID	> 3 m < 6 m	> 6 m	Total no. dives	Dates	Hours monitored
	B	49	51	2999	1-31	57 * 6hr
	C	44	56	357	28-31	4 * 6hr
Aug	E	49	51	738	7-31	29 * 6hr
	G	54	46	1512	16-27	33 * 6hr
	H	64	36	2036	18-31	27 * 6hr
	B	7	93	1044	13-30	20 * 6hr
Sep	E	36	64	243	1-19	9 * 6hr
	H	47	53	1688	1-30	91 * 6hr

Table 13 Comparison of the means of the "daily maximum dive depth" values of 3 adult male Atlantic walruses in August and September 2000 in NE Greenland. Period 1 corresponds to the duration of the deuterium dilution measures in August (from first enrichment to final sampling). Period 2 corresponds to the following period in August and September 2000 where the animals were undisturbed. Values for dives during the deuterium oxide experimental period and outside this in August and September are presented. Data were Ln-transformed for the t-tests.

	Period 1				Period 2						
ID	Mean (m)	SD	min-max (m)	n	Mean (m)	SD	min-max (m)	n	t-value	p	df
B	47	22	12-72	7	74	60	6-170	10	-0.24	0.81	14
E	75	48	36-128	3	57	50	24-132	4	0.69	0.52	5
G	8	0	8-8	2	36	26	12-84	9	2.32	0.05	9

Effect of activity on water turn-over

Both water turnover rate and dive activity information was available for 6 animals. In none of the animals was there a clear correlation between water turnover rate (WTR) and average dive duration ($R^2 = 0.45$, $p > 0.05$), % of time spent below 6 m ($R^2 = 0.35$, $p > 0.05$) and with the number of dives deeper than 6 m ($R^2 = 0.22$, $p > 0.05$). Furthermore, there was no correlation between WTR and either % of time hauled out ($R^2 = 0.02$, $p > 0.05$) or average dive depth ($R^2 = 0.01$, $p > 0.05$).

Discussion

This study was a field investigation of the water metabolism of walruses in view of future IDA studies (Doubly Labeled Water and Body Composition) where isotope dosage depends heavily on the size of the dilution space and on the importance of the water turnover.

Total Body Mass

The precision of the TBM values for each study subject is essential for the accuracy of the body water and body composition calculations. The values of TBM were calculated from direct measures of standard length and axillary girth (American Society of Mammalogists 1967) according to the allometric equations for Atlantic walruses of Born *et al.* (2003) which revised and improved the equations of Knutsen and Born (1994) by including additional three directly weighed animals. The equations of Born *et al.* (2003) have a $r^2 = 0.99$ and are therefore considered a good predictor of body weight from measures of body size.

Background enrichment of the isotope

Variations of background levels of the isotopes used for enrichment can influence the results of the analysis and have to be taken into careful consideration (Speakman 1997). In this study it was chosen to sample venous blood of each animal immobilized in order to obtain an individual reference point for further calculation. During the 2001 season, seawater was also sampled regularly simultaneously with walrus blood. The results of the analysis indicate small and statistically non-significant daily fluctuations of both the environmental and the animal background levels. However, there was a significant difference between blood background levels between the two sampling years. This observation confirms the importance of measuring the background isotope concentration during the experimental period.

Isotopic equilibration

If the isotope is administered intravenously and blood is sampled before complete equilibration there is a risk to underestimate TBW. Generally in pinnipeds equilibration times range from 0.5 – 3.0 hr (Bowen and Iverson 1998). Prior to the current study an isotope equilibration experiment for a single walrus has been conducted (Lydersen et al. 1992a). A 960-kg walrus was enriched with an intravenous injection of tritium oxide and venous blood was sampled regularly for 5 hr following the bolus injection. Observations indicated that equilibration was reached already after 1 hr after the injection of the isotope. Speakman (1997) suggested that the short equilibration time observed by Lydersen *et al.* (1992a) was caused by the intravenous injection of the isotope followed by blood sampling from the same body water pool in a multi-pool model and therefore that complete isotopic equilibrium in the body was not achieved. An equilibration time of 1 hr is surprisingly short as equilibration times longer than 1 hr have generally been observed in large animals

(Bowen and Iverson 1998). Differences in blood flow distribution in the different body compartments have been identified as the possible cause of this long equilibration time (Coleman et al. 1972). In the present study, venous blood was sampled regularly for up to 6 hr post intravenous bolus injection. The results indicate that the equilibration time for walruses administered an intravenous bolus of isotope is approximately 2-3 hr and this value was adopted in the calculations of TBW.

Total Body Water

One of the important assumptions when using IDA is that the administered isotope mixes completely and exclusively with the body water pool of the subject studied. In practice some of the isotope is lost to other body components such as the rapidly exchangeable hydrogen atoms in the organic constituents of the body (Ussing 1935). The dilution space can be overestimated if the experimental animal has a full digestive tract because the water molecules contained in the intestinal lumen are highly exchangeable with the body water molecules (Speakman 1997). However, most of the animals in this study had hauled out prior to enrichment and presumably their digestive tract was empty. Hauled out walrus usually have empty stomach and intestine (Fay 1982). Only a proper validation study involving isotopic enrichment followed by carcass desiccation of the same individual can fully assess the magnitude of this overestimate for the species in question. Based on several studies Bowen and Iverson (1998) developed a general regression equation for the overestimation of TBW from desiccation by hydrogen IDA. This equation can be used to estimate TBW from H-dilution space for those pinnipeds for which validation data are not available. In absence of a true validation study, the present work adopted the above approach to estimate TBW. The TBW estimate by desiccation is also an approximation that might underestimate the “real” TBW because of evaporation or incomplete drying, but it is generally assumed as the reference value. Relating all measures to TBW by desiccation is useful because it provides a consistent basis for comparative studies (Bowen and Iverson 1998).

Water Turnover Rate

In this study it was possible to measure WTR in 8 cases (the WTR for two individuals was measured twice over the two seasons). Nagy and Costa (1980) have critically reviewed the relative importance of the assumptions listed by Lifson and McClintock (1966) which have to be valid in order to obtain reliable estimates of water flux from IDA methods. One of the assumptions is that body water remains constant during the measurement period (Nagy and Costa 1980).

In this study it was assumed that the body water pool remained constant throughout the measurement period. However, one animal was subjected to a second enrichment followed by a sufficient equilibration period at final recapture. The double equilibration allowed an estimate of TBW both at the beginning (630 kg) and at the end (746 kg) of the measurement period. This represents an increase in TBW from 50% to 60% of TBM and indicates that the TBW content of a walrus may actually change considerably with time. Changes in

TBW for a free living and freely foraging animal can be due to exercise and foraging activity with consequent change in body condition/composition. For this animal it was possible to improve the calculation of WF using the equations in Nagy and Costa (1980).

Body Composition

The blubber layer is the major energy store used by pinnipeds (Bryden 1968) and it is often used to assess body condition in seals (Gales and Renouf 1994; Gales et al. 1994a). Non-destructive methods for assessing body composition are obviously desirable in conservation studies. IDA provides the possibility of estimating TBW and hence TBF according to relationships established for other mammalian species (Pace and Rathbun 1945; Reilly and Fedak 1990). However, an initial validation of the general allometric equations is necessary. In the case of field studies of pinnipeds it is often logistically difficult to carry out accurate proximate body composition analysis. For this reason in this study, the body composition estimated from IDA has been compared with the data obtained from *in situ* dissection of the Atlantic walrus that were killed during the traditional walrus hunt by the Greenland Inuit (Knutsen and Born 1994). The comparison of the two data sets suggests that both the simple model of Pace and Rathbun (1945) and the specific model of Reilly and Fedak (1990) provide a useful approximation for walruses. The statistically non-significant small difference between the mean blubber content obtained in Knutsen and Born (1994) and the mean value from IDA could be ascribed either of two factors. Firstly blubber content obtained from dissection is necessarily an underestimate of the actual TBF value as blubber represents the most important but not the only body fat deposit. Furthermore, the method of physically separating blubber from the carcass used by Knutsen and Born (1994) implies a certain, though presumably minimal, loss of material. Secondly the values in Knutsen and Born (1994) refer to animals sampled in spring when the animals are leanest (Fay 1982).

The correspondence of the estimates of TBF in animal H as obtained from IDA and dissection confirmed of the validity of IDA estimates compared to direct weighing. It was not possible to compare the results of the application of the allometric equations for gray seals on the other body components, but the values are presented here as reference.

It has been mentioned previously that a full digestive tract can lead to overestimation of TBW. This could be one explanation for walrus M's apparently high hydration level. However, it must be pointed out that a correction of the allometric equations for TBF in gray seals might be necessary in order to apply them to walrus as gray seal blubber has been analyzed to contain 9.1 – 35.9% water (Reilly and Fedak 1990), while walrus blubber has been reported to contain 23 - 32% water (Kuhnlein et al. 2002).

Finally, the results of a study on southern elephant seals indicate that morphometric modeling derived from surface area, mass, length and girth combined with IDA can accurately estimate TBW (Tierney et al. 2001). These results suggest that a similar relationship might be valid for other pinnipeds, hence walruses. Further concurrent measures of body composition by IDA and morphometric measures would in this case allow the development of a reliable predictor for body condition for this species to be used in field studies without the need of long immobilization times necessary for IDA studies.

Activity levels in the experimental period

Although clearly some individual variability in overall activity existed, it is likely that the diving activity of the treated animals had not been abnormally influenced by the handling procedures to which the animals had been subjected. Dive depth was not markedly different during the experimental period compared with the inshore situation in September when the animals were not subjected to any immobilization procedure (Table 13). Studies of walrus activity by use of SLRT indicate that the individual time allocation between resting and diving of walrus during August and September may vary with a gradual decrease in hauled out time in September (Born and Acquarone *this thesis*). In this study it was not possible to study haulout behavior comparatively between the two periods, however, there is an indication of increased dive depth and of the duration of the dives in September compared to August (Table 11 and Table 12).

The data available in this study don't indicate any correlation between the observed changes in activity levels and the body water flux. It is possible that the lack of correlation is due to the paucity and heterogeneity of data, however, it is more likely that the behavior of the studied animals was not markedly different during the two periods (e.g. feeding activity and energy expenditure).

It is suggested to assume the values of water content, turnover and flux in this study as a general measure for summering male walruses and that they be utilized as a basis for a proper validation study of the water and energy physiology of walruses.

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Paper IV: Direct measures of pinniped field metabolic rate: implications for fisheries models

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Abstract

The food requirements of free-ranging pinniped species can be derived from measures of Field Metabolic Rate (FMR) by the Doubly Labelled Water (DLW) method. However, fisheries models typically rely on indirect estimates of pinniped food requirements based on their theoretical basal metabolism multiplied by 3-4. Reluctance to employ direct measures of metabolism might be due to the limited body-size range of the seven pinniped species for which DLW studies are available to date. The measure of FMR of walruses in this study extends this range by a factor of 10. It also allows the derivation of a predictive equation for pinniped FMR [Ln-FMR (MJ/day) = 0.173 + 0.816 Ln-Total Body Mass (kg)] and it suggests that pinniped food requirements might be double as high as assumed in fisheries models.

Keywords: food requirements, metabolic rate, feeding rates, pinnipeds, marine mammals, doubly labelled water; *Odobenus rosmarus*

Introduction

Conflicts between fisheries and marine mammals have escalated and are likely to increase during the next century (DeMaster *et al.* 2001). Fisheries models for evaluating the impact of marine mammal predators on fish stocks require accurate estimates of food intake rates (Innes et al. 1987; Trites et al. 1997; Bowen 1997; Bjorge et al. 2002; Winship et al. 2002). Field energy demands (generally called Field Metabolic Rate or FMR) and thus food consumption rates of free-ranging marine mammals have been estimated from their heart rate (Boyd *et al.* 1999) or by multiplying their inferred basal metabolic rate (BMR) by some factor (BMR x 2-3) (Lavigne et al. 1986; Innes et al. 1987). However, heart rate monitoring is a valuable method for estimating metabolic rates, but it requires species-specific validation of the relationship between heart rate and metabolic rate and it may not accurately reflect metabolic rates during digestive events (McPhee *et al.* 2003). Furthermore the adaptations of marine mammals to diving, e.g. bradycardia (Elsner 1999), may complicate its interpretation. Finally, the use of BMR to estimate food consumption of marine mammals is problematic, as the conditions required for the measurement of BMR were established for terrestrial animals (White and Seymour 2003) and may be inappropriate for marine mammals.

An alternative approach is to calculate the energy demands from CO_2 production, measured from the differential elimination of two isotopic tracers in body water – known as the Doubly Labelled Water (DLW) technique (Speakman 1997; Costa and Gales 2003). This technique avoids some of the problems associated with the other methods, and the resultant FMR measurement can be incorporated directly into fisheries and other ecological models. A problem with the DLW technique is the high cost of the isotopic labels, which increases exponentially with body mass. For this reason the 7 pinniped species for which DLW estimates of FMR have been derived are far lighter than the largest species (Reeves *et al.* 1992) and ranged only between 27 and 114 kg (Nagy et al. 1999; Costa and Gales 2003). A reliable allometric equation for FMR versus body mass (BM) can be generated for animals within this BM range (Nagy *et al.* 1999), but the predicted estimates for larger animals are potentially inaccurate due to extreme extrapolation, which has perhaps contributed to the reluctance of modellers to include DLW measurements into fisheries model calculations.

In this study we used the DLW method to estimate the FMR of two free-ranging male walruses. The body mass of the two animals in this study extends by 11 fold the mass of the previous largest pinniped measured by the DLW method (and they are by far the largest animals studied using this methodology). These estimates extend the validity of the allometric equation for pinniped FMR across most of the body size range of pinnipeds.

Materials and Methods

Study site and animals

The study animals, all adult male Atlantic walruses (*O.r.rosmarus*) (Table 14), were chosen from an all-male group on a terrestrial haulout site in NE Greenland at 76°52.8'N, 19°37.9'W (Born *et al.* 1995).

In August 2001, two animals were enriched with DLW. Before handling, they were completely immobilised (Born and Knutsen 1992a). During immobilisation the animals’ axillary girth and standard body length (Committee on Marine Mammals 1967) were measured for estimation of TBM (Knutsen and Born 1994; Born et al. 2003), a satellite radio and a dive recorder were attached to the tusks and venous access was gained by catetherization of the epidural vein in the lumbar region for isotope enrichment and blood sampling. Upon recapture a similar immobilisation procedure was used, the size measures were repeated along with blood sampling and instrument data retrieval.

For comparison in August 2000 (3) and 2001 (1) four other animals were also instrumented with a satellite radio and a dive recorder to obtain behavioural data.

Table 14 Activity of six adult male walruses in North-east Greenland during August 2000 and 2001.

	Mass	Days monitored	Time hauled-out	Dives/day	Mean dive duration	Mean depth	Max depth
ID	kg	n	%	n	min (SD)	m (SD)	m
A	1,370	5.0	27.2	108	3.5 (2.1)	12.6 (5.5)	55
B	1,250	8.7	27.2	133	4.4 (2.1)	15.8 (10.5)	145
C	1,546	14.9	41.1	208	4.8 (1.8)	14.9 (12.9)	192
D	1,115	12.0	47.8	170	5.1 (1.4)	12.1 (5.1)	84
E	1,086	7.2	34.5	200	4.9 (1.1)	11.3 (4.2)	51
F	1,284	12.0	20.0	170	5.5 (2.2)	12.3 (7.6)	189

Energy expenditure

At initial capture, the two designated animals' venous blood was sampled through the catheter for determination of background isotope concentration. Each animal was subsequently administered an intravenous dose of 97.75 g of deuterated water, 43.9% ^2H_2O (Merck 1.13366, E.Merck, D-6100 Darmstadt, Germany), and 157.62 g of ^{18}Oxygen enriched water, 41.5% $H_2{}^{18}O$ (Rotem Industries Ltd., P.O.Box 9046, Beer-Sheva 84190, Israel). A series of blood samples was taken at approximately 30-minute intervals for 4 hours for determination of the isotope equilibration curve, and isotope dilution spaces. Animal A was enriched on the 16 August 2001 at 16:42 and recaptured on the 21 August 2001 at 17:52. Animal B was enriched on the 7 August 2001 at 21:08 and recaptured on the 16 August 2001 at 15:30. Immediately after sampling whole blood was stored in 2.0 ml standard glass vials and flame sealed into 100 µl precalibrated glass pipettes (Modulholm A/S, Vasekaer 6-8, DK-2730 Herlev, Denmark, VITREX model 1272). Seawater background samples were collected and sealed in 2.0-ml glass vials throughout the experimental period to investigate variation in environmental isotope enrichment. All samples were stored at ambient temperature (max. 3°C) while in the field and were subsequently kept at 5°C prior to analysis.

All blood samples were vacuum distilled into Pasteur pipettes (Nagy 1983) and the distillate was used for determination of both ^{18}O and ^2H concentration. For ^2H-analysis H_2 gas was produced by reduction with excess $LiAlH_2$ as described in Ward et al. (2000) For ^{18}O-analysis 10 µl of distillate was measured using the small sample equilibration method (Speakman *et al.* 1990). The isotopic composition of the injectate was measured by diluting a weighed quantity of the injectate (0.1-0.2 ml) into a weighed quantity of tap water (60 ml). This mixture was then treated in exactly the same manner as the distillate from the blood samples. In each batch of samples for analysis, laboratory standards were included to account for day-to-day variation in the analyser. All isotope enrichments were measured in δ-units and converted to ppm using the established ratios for reference materials. We evaluated precision of the derived estimate of CO_2 production using the iterative procedures in Speakman (1995), and converted the mean estimate to metabolic rate assuming an RQ of 0.85. Calculations were made using the DLW program (version 1.0, Speakman and Lemen, Naturware, 1999).

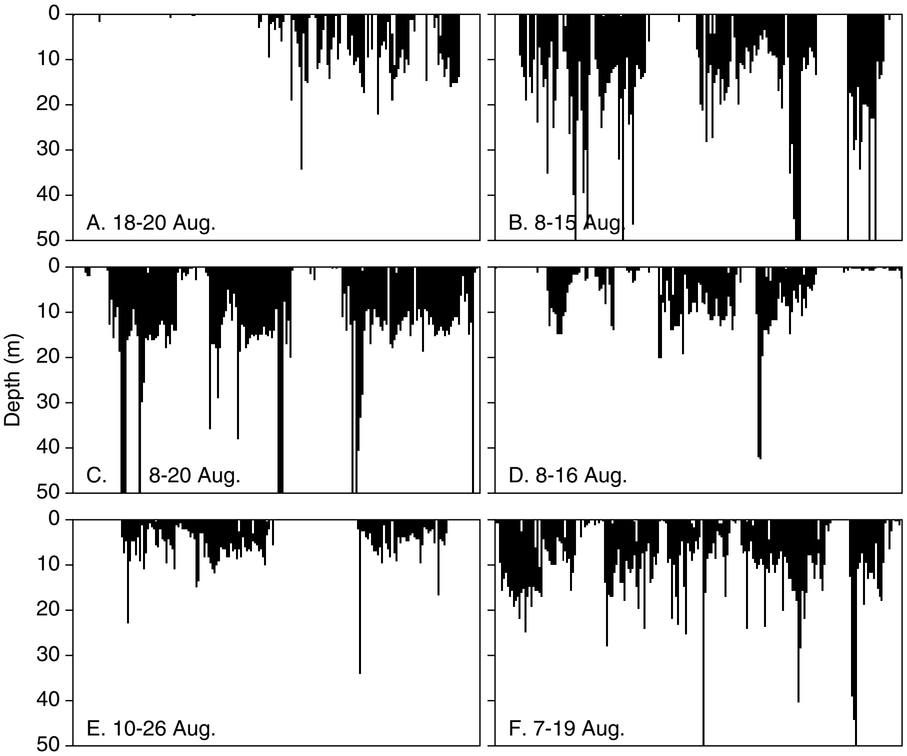
Activity of the animals

The two study animals and four other walruses were instrumented with satellite linked radio transmitters and dive recorders to obtain data on movement, haulout and dive activity (Table 14).

An ARGOS System SPOT2 satellite-linked radio transmitter with "time at temperature" histograms and a MK7 Time Depth Recorder (TDR) with 500 m range (Wildlife Computers, 16150 NE 85th Street - Suite 226, Redmond, Washington 98052 USA) were attached to each of the tusks of six adult male walruses using the method in Born and Knutsen (1992b). The TDR's were programmed to sample depth, temperature, and light level at intervals of 5, 300 and 300 or 15, 600 and 120 seconds respectively. The GIS software ArcView 3.2a was used for calculation of the horizontal movement of the walruses after satellite-telemetered locations of all quality classes had been run through a PC-SAS®ARGOS-filter (V.5.0, D.Douglas USGS, Alaska Science Center, 100 Savikko Road, PO Box 240009, Douglas, AK 99824, USA, unpublished method).

The TDR data were analysed using the software provided by the manufacturer (the Zero-Offset-Correction and Dive-Analysis). Periods when the walruses were hauled out on land or ice were excluded from the analysis of dive activity. Minimum depth for dives to be analysed and maximum depth to be considered at surface were set to 6 m. The time spent at sea or out of the water was determined by analysing the temperature record of the TDR, where only temperatures below 2.5°C were considered as coming from a submerged sensor. Number of dives, dive duration, surface times were also determined for each individual.

Figure 12 Dive profiles by Time-at-Depth recorders of six adult male walruses in North-east Greenland in August 2000 and 2001 (Table 14).



Results

Animals spent on average 33.0% of their time hauled out which is typical of walruses during summer (Born and Knutsen 1997). Diving activity accounted for 50.8% of the time spent at sea, with an average rate of 165 dives per day with a duration of 3.5-5.5 min (Figure 12 and Table 14). Although the time spent hauled out by the two DLW animals was similar, B was diving more actively than A as indicated by the number of dives per day, the mean dive duration and dive depth and the maximum depth reached (Table 14).

A previous study (Lydersen *et al.* 1992) had suggested that isotopes (tritium) in walruses might equilibrate within 1 hour. We found however that equilibration time of the isotopes took approximately 2.5-3.0 h. We therefore used these estimates of the initial isotope enrichment combined with the recapture samples to estimate field metabolic rate.

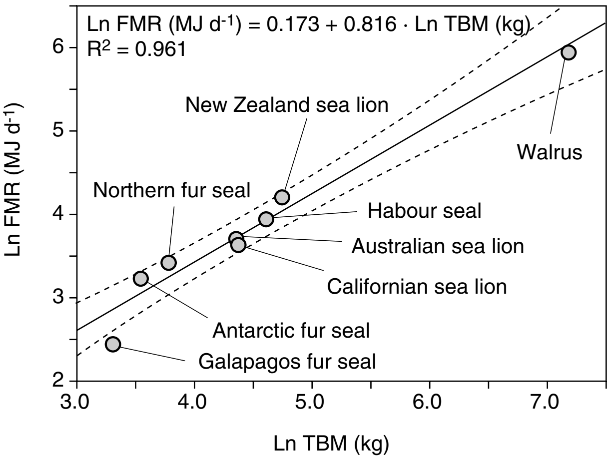
Body water (BW) percentage of body mass from dilution of the oxygen isotope was 45.0% in A, and 49.5% in B. The lower BW content of the larger A suggested that it had relatively more blubber. The estimated FMR's were 345.0 (SE 7.5) MJ/day for A and 417.4 (SE 6.2) MJ/day for B, using the single-pool model for calculation (Lifson and McClintock 1966) (mean = 381.2 MJ/day). Using the two-pool model (Speakman 1997) and the mean observed dilution space ratio (Schoeller *et al.* 1986) (1.09), the corresponding estimates were 328.1 (SE 8.7) MJ/day and 365.4 (SE 15.4) MJ/day, respectively (mean = 346.8 MJ/day). A best fit relationship between FMR and BM including only all previous DLW-studies of pinnipeds (Table 15; Lifson and McClintock (1966) single-pool calculation) explained 88.3% of the variation in FMR. The direct estimate of FMR in the present study was about 43% lower than that predicted by this relationship clearly indicating the need for a more precise equation for larger pinnipeds. The new allometric equation [Ln-FMR (MJ/day) = 0.173 + 0.816 Ln-Total Body Mass (kg)] for pinniped FMR in this study explained 96.1% of the variation (n=8 species) (Figure 13). Including data on diving behaviour and activity did not improve this relationship.

Table 15 Average body mass and field metabolic rate by doubly labelled water in eight species of pinnipeds. References: 1-Costa & Gales 2003, 2-Nagy *et al.* 1999, 3-this study.

Species	Scientific name	BM (kg)	FMR (MJ/d)
Galapagos fur seal[1]	*Arctocephalus galapagoensis*	27	11.7
Antarctic fur seal[1,2]	*Arctocephalus gazella*	34	25.7
Northern fur seal[1,2]	*Callorhinus ursinus*	43	30.6
Australian sea lion[1,2]	*Neophoca cinerea*	76	40.9
Californian sea lion[2]	*Zalophus californianus*	78	38.6
Harbour seal[2]	*Phoca vitulina*	99	52.5
New Zealand sea lion[1,2]	*Phocarctos hookeri*	114	68.0
Walrus[3]	*Odobenus rosmarus*	1,310	381.2

Environmental background isotope enrichments measured in sea water did not fluctuate significantly during the study period and did not differ significantly from the background enrichments in the animals' blood collected prior to injection.

Figure 13 Field metabolic rate (FMR) in relation to body mass (BM) in eight different pinniped species based on measurements using doubly labelled water (actual data in Table 15). 95% confidence intervals of the regression are shown as dashed lines.



Discussion

A FMR of 381 MJ/day for a 1300 kg walrus as measured in this study, corresponds to the consumption of about 83 kg food per day (fresh matter) calculated from the mean energy composition of the walrus prey items from East Greenland (Born *et al.* 2003). This value is well within previously estimated range of 42-92 kg food intake for free-ranging walruses weighing 1100-1200 kg (Fay 1982).

The greater FMR value of B may have been due to its higher diving activity (Table 14). Haul-out time for all animals measured by TDR in this study was on average 33% which is higher than 30% previously reported from this area (Born and Knutsen 1997) 26% from Alaska (Hills 1992) and 26% from Svalbard (Gjertz *et al.* 2001). However for the same areas variability in haul-out time between individuals can be considerable (Born and Knutsen 1997; Gjertz et al. 2001).

The single pool equation of Lifson and McClintock (1966) to derive FMR was used here for consistency with the previous studies, but this equation over-estimates energy demands for animals that are larger than 5-10 kg (Speakman 1997). A two-pool model calculation is probably more appropriate. Since most papers do not quote the necessary parameters to make recalculations, we were unable to construct a prediction based on the two-pool method. However, our estimates, and those of Costa and Gales (2003), indicate that the overestimate using the single-pool method (Lifson and McClintock 1966) might only be 9-17% (averaging 13%).

Current fisheries models that have utilised estimated daily food consumption predicted from multiples of BMR [predicted from body mass using the Kleiber equation (Kleiber 1932; 1961)] have routinely assumed that the FMR of pinnipeds is around 3 x BMR (Trites et al. 1997; Nilssen et al. 2000; Bjorge et al. 2002; Winship et al. 2002). Our study, along with the other DLW studies contributing to the derived equation, however, suggests that this is a serious underestimate of pinniped food intake. FMR's derived from the equation in this study average between 5.5 (for a 100-kg seal) and 6.5 (for a 1300-kg seal) times the Kleiber BMR prediction. Using these direct estimates of FMR would more than double the estimated daily food requirements of pinnipeds and their projected impacts on prey species. Consequently, many current fisheries models may seriously underestimate the impacts of marine mammal predators on fish stocks.

The allometric equation for pinniped FMR derived here can be utilised to revise the impact of pinnipeds on fish stocks in fisheries models, since it provides a mass-specific prediction of FMR for most species without the need for extrapolation. Most importantly, it is based on *direct* measurements of FMR rather than inferences from multiples of basal metabolism.

The costs of DLW preclude its routine use in studies of the energetics of larger pinniped species such as the walrus. Nevertheless, the current study has demonstrated that occasional measurements of FMR can improve and refine the assumptions that underpin models being used to assess levels of competition between seals and fisheries.

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Paper V: Underwater observations of foraging free-living Atlantic walruses (*Odobenus rosmarus rosmarus*) and estimates of their food consumption

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ORIGINAL PAPER

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Underwater observations of foraging free-living Atlantic walruses (*Odobenus rosmarus rosmarus*) and estimates of their food consumption

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Abstract Food consumption of Atlantic walruses (*Odobenus rosmarus rosmarus* L.) was quantified by combining underwater observations of feeding with satellite-telemetry data on movement and diving activity. The study was conducted between 31 July and 7 August 2001 in Young Sound (74°N–20°W) in Northeast Greenland. On ten occasions, divers were able to accompany foraging walruses to the sea floor and collect the shells of newly predated bivalves (*Mya truncata*, *Hiatella arctica*, *Serripes groenlandicus*) for determination of number of prey and biomass ingested per dive. Simultaneously, the activity of a 1,200-kg adult male walrus was studied by use of satellite-telemetry during an entire foraging cycle that included 74 h at sea followed by a 23-h rest on land. An average of 53.2 bivalves (SE = 5.2, range: 34–89, $n = 10$) were consumed per dive, corresponding to 149.0 g shell-free dry matter (SE = 18.9, range: 62.4–253.1 g), or 2,576 kJ per dive (SE = 325.2, range: 1,072–4,377 kJ). During the foraging trip, the walrus spent 57% of the time diving to depths of between 6 and 32 m, and it made a total of 412 dives that lasted between 5 and 7 min (i.e. typical foraging dives). If the entire feeding cycle is considered (97 h), the estimated daily gross energy intake was 214 kJ per kg body mass (95% CI: 153–275 kJ), corresponding to the ingestion of 57 kg (95% CI: 41–72 kg) wet weight bivalve biomass per day, or 4.7 (95% CI: 3.3–5.9%) of total walrus body mass. Due to ice cover, walrus access to the plentiful inshore bivalve banks in the area is restricted to the short summer period, where walruses rely on them for replenishing energy stores. It is hypothesised that the documented decrease in the extent and duration of Arctic sea ice may increase food availability for walruses in eastern Greenland in the future.

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Introduction

Walruses (*Odobenus rosmarus*) are highly specialised predators and mainly consume bivalves (Fay 1982). Walruses move inshore after break-up of the land-fast ice to exploit the shallow-water mollusc banks during the short open-water season (Born et al. 1997). Although types of prey are well known, the amount of food consumed per day by free-living walruses is not. Quantitative information of this kind is necessary to obtain a reliable prediction of the effects on population size of future changes in sea-ice cover.

Although walruses may feed on a variety of bottom-dwelling invertebrates, only a few bivalves—usually *Mya* sp., *Hiatella* sp. and *Serripes* sp.—make up the bulk of their diet (Vibe 1950; Fay 1982; Fay et al. 1984; Sheffield et al. 2001). The need for quantitative information on the feeding of walruses has become increasingly apparent in recent years. Major climatic changes have influenced the sea-ice cover in those areas of the

Arctic (e.g. Rothrock et al. 1999; Parkinson 1992, 2000) where small and still highly vulnerable sub-populations of Atlantic walruses (*O. r. rosmarus* L.) live (reviewed by Born et al. 1995). In the eastern-Atlantic Arctic, including the East Greenland and Svalbard areas, the ice cover has decreased in both thickness and extent during the 1990s (Rothrock et al. 1999; Parkinson 1992, 2000). Temperatures have increased in the East Greenland-Svalbard area since the 1960s (Førland et al. 2002; Hanssen-Bauer 2002). In eastern Greenland, a small and genetically distinct group of walruses that apparently was on the verge of extinction in the first half of the twentieth century shows encouraging signs of increase (Born et al. 1995, 2001; Andersen et al. 1998). However, the East Greenland sub-population is thought to still number only about 1,000 animals (Born et al. 1997). To be able to determine how the dynamics of this group of walruses will be influenced in the future by the changes in ice cover, quantitative data on individual foraging rates of free-living walruses are needed.

Foraging rates of free-living walruses have been inferred from amounts of stomach content (Vibe 1950; Fay 1982; Fay et al. 1984), records on the sea floor of predation by walruses (Oliver et al. 1983), visual at-surface observations of foraging animals (Fay 1982; Born and Knutsen 1997), and information on diving activity of individual walruses fitted with time-depth recorders (TDRs) (Wiig et al. 1992; Gjertz et al. 2001; Jay et al. 2001) and satellite-linked radio transmitters (Born and Knutsen 1992, 1997; Gjertz et al. 2001). Studies of food and energy intake and growth of captive Pacific walruses (*O. r. divergens* Illiger) were made by Fisher et al. (1992) and Kastelein et al. (2000), whereas estimates of food and energy consumption of free-living walruses have been based on a combination of information on feeding in captivity and samples of stomach content in the wild (Fay 1982; Genrich 1984 *fide* Fisher et al. 1992).

In the present study, data on the foraging rate in free-living Atlantic walruses in northeastern Greenland during summer are presented. Information on number of bivalves consumed per dive obtained by underwater observations are combined with satellite-telemetry data on movement and activity of an adult male walrus during an entire feeding excursion that was made in the same area and simultaneously with the observations of foraging. On the basis of these data, an estimate of the food and energy intake of a free-living walrus during a typical summer foraging event is derived. The estimate of food consumption is related to the biomass and productivity of inshore bivalve communities.

Materials and methods

Study site

The study was carried out in August 2001 in Young Sound (74°18'N 20°13'W) (Fig. 1), a fjord of about 90 km in length and 2–7 km in width with a 40- to 50-m-deep sill at the entrance. The study site, which encompassed the outer parts of the sound, is minimally influenced by discharge from glaciers, but pulsed terrestrial run-off occurs during the short summer thaw generating an estuarine circulation and ensuring an efficient water exchange with the Greenland Sea (Rysgaard et al. 2003). Sea ice covers the fjord for 9–10 months of the year. During summer—or the open-water period—a low-salinity (25 psu), 2–4°C surface layer (0–5 m) is present. Below this layer, salinity increases to >32 psu with subzero temperatures below 15–20 m (Rysgaard et al. 1999). Average air temperature during July/August may reach 3–4°C with mean wind velocities of 4–5 m per second (Cappelen et al. 2001). For further details on the study area, see Rysgaard et al. (2003).

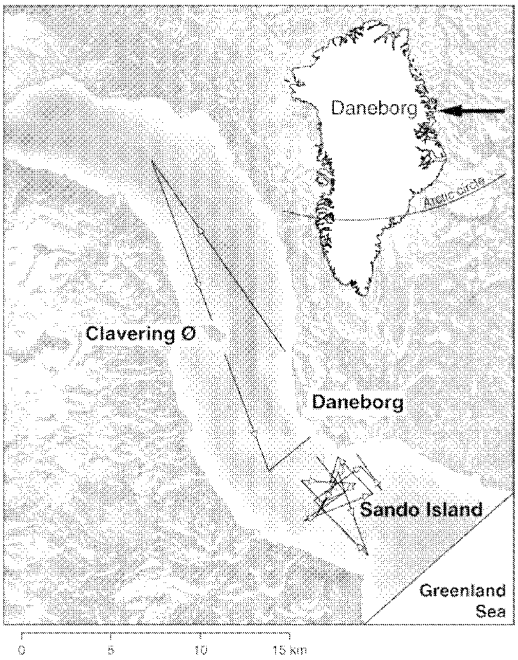

Fig. 1 The Young Sound study area (NE Greenland) with tracks of an approximately 1,200-kg adult male walrus during a 74-h feeding trip (2–6 August 2001) that began and ended at the terrestrial haul-out on Sando Island. *Light shading* in the fjord indicates shallow-water areas

The macrofauna community in Young Sound is well developed and exhibits high species diversity. Bivalves (primarily *Mya truncata* L., *Hiatella arctica* L. and *Astarte* spp.) dominate the macrozoobenthos at depths of 10–40 m with biomasses of up to 200 g wet weight/m^2. Brittle stars dominate at greater depths where they may reach an abundance of up to 269 individuals/m^2 (Sejr et al. 2000).

From break-up of the solid fjord ice in mid-July to the formation of new ice around mid-October, a group of up to about 50 walruses—primarily adult males—haul out on the southern tip of the island of Sando (Fig. 1) (Born et al. 1997). Females are very scarce in these areas and therefore our study deals only with the foraging of adult males during the inshore period.

Observations of diving activity

Between 26 July and 20 August 2001, systematic observations of walruses were made from the mainland at Daneborg (Fig. 1) within a semicircular area with a radius of about 4 km from a post 45 m above sea level (74°18'443"N–20°13'006"W) and about 400 m from the coast line. The purpose was: (1) to obtain systematic data for a quantification of foraging activity of walruses in the Young Sound study area, and (2) to keep track of individual walruses and identify circumstances in which it would be possible to dive alongside them to collect data on foraging.

Track was kept of all walruses that appeared within the study area, and their diving and swimming behaviour was noted systematically. The locations of sites where walruses surfaced and dived were determined by use of a theodolite (Wild GST 20) according to methods described in Würsig et al. (1991). A total of four to six 2-h watches were kept around the clock with a "rolling" schedule, which ensured that all hours were surveyed twice during the entire study period. Observations were usually suspended when the sea state exceeded Beaufort 2, in which case it was no longer considered possible to detect and observe walruses reliably at any distance. A Svarowski binocular telescope (x30 and x75 magnification), a (Kowa SN-1) Svarowski monocular telescope (x30–60 magnification) and hand-held binoculars (x7 magnification) were used during the observations.

A sub-set of observations, consisting of ten occasions where walruses were studied prior to, during and after the presence of divers, is presented here.

Five male walruses that were immobilised and handled on Sando Island in 2001 (Fig. 1) had total body masses (TBM) ranging from 900 to 1,400 kg (E.W. Born, unpublished data). These animals were typical of the group that uses the terrestrial haul-out and also of the walruses that were observed foraging in Young Sound.

Collection of data on walrus predation on bivalves

Whenever a foraging walrus had been tracked for a certain amount of time during favourable sea and weather conditions at a location where the water depths were not too great for scuba divers to reach the sea floor safely, a team of divers was notified. The divers approached the walrus slowly in a rubber dinghy powered with a 40-hp outboard engine. In Northeast Greenland, walruses are fully protected and are not hunted inside the National Park of North and Northeast Greenland (cf. Born et al. 1997). They are usually not afraid of humans, therefore, but when approached for the first time some walruses did become nervous and dived away from the dinghy. After the approach had been repeated 2–5 times, however, most individuals accepted the presence of the dinghy and it was possible to get within a distance of about 10 m without disturbing their feeding cycle. One or two divers then entered the water and swam at the surface to a distance of 1–5 m from the ventilating animal. When the walrus dived, the divers followed it to the sea floor where they documented its feeding behaviour by filming a complete foraging bout (Fig. 2A). During a subsequent dive, empty bivalve shells were collected at the feeding pit and examined in the field laboratory (Fig. 2B). Shells of newly predated bivalves were easily recognisable in that they still contained some soft parts (2–3%) uneaten by the walrus and not yet removed by amphipods, gastropods and other scavengers (Fig. 2C).

Determination of bivalve biomass and energy contents

Shell lengths of all collected bivalves containing soft parts were recorded using a digital caliper. Relationships between mass of shell-free dry matter (g SF DM) and shell length (mm) were available for *H. arctica* (Sejr et al. 2002) and *M. truncata* (M.K. Sejr, unpublished work): *M. truncata*, $\text{mass} = 0.000005\ \text{length}^{3.45}$, $n = 106$; *H. arctica*, $\text{mass} = 0.00002\ \text{length}^{2.635}$, $n = 108$. In the case of *Serripes groenlandicus*, isometric growth was assumed and the theoretical weight exponent of 3 was used according to King (1995). For 15 *H. arctica* and 15 *M. truncata* individuals, the relationship between dry weight, organic carbon content and wet weight was determined. Soft parts were dissected out and blotted dry with paper towels before weighing. Samples were then dried at 105°C for 24 h to obtain shell-free dry weights. Organic-carbon content was determined on dry homogenised tissue weighed into

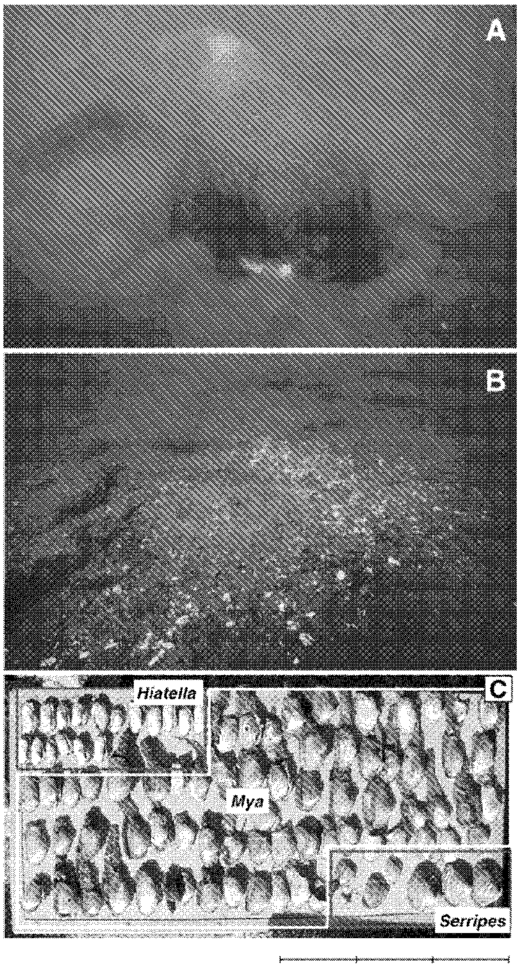

Fig. 2 A An adult male walrus rooting with its vibrissae for bivalves in the bottom sediments of Young Sound (NE Greenland). After a walrus had located a clam, it sucked out the soft parts and left the shells behind. **B** A recently used feeding patch (diameter = ca. 2 m) resulting from a single foraging dive. Shells from old and newly predated bivalves surrounded the patch, which could be distinguished from the surrounding sediment by its light colour. While the walrus was feeding, a diver marked the exact location of the feeding patch by placing a block of lead attached to a streamer that reached the surface. After a foraging dive, the diver waited for about 10 min for disturbed sediment to settle before collecting all bivalves (living and dead) from the feeding patch. Hence, records of the foraging dive were sampled less than 20 min after it ended. **C** Shells, with siphon sheath and other remnants of soft parts still attached, from 89 bivalves (3 species) that were ingested by a walrus during a single dive at a depth of about 13 m on 31 July 2001 (scale bar, 30 cm)

sample boats. Analyses were performed on a C/N elemental analyser (RoboPrep-C/N, Europa Scientific, UK). In the case of *S. groenlandicus*, too few individuals could be collected to establish a relationship between shell length and dry weight. To convert dry weight into wet weight, mean values of the conversion coefficients calculated for *H. arctica* and *M. truncata* were used. Energy contents in 10 *M. truncata* and 15 *H. arctica* were determined by bomb calometry according to methods described in Fisher et al. (1992). The energy content of *S. groenlandicus* was not determined but was assumed to be an unweighted average of those of the two other species.

Satellite tracking

In order to determine individual movement and diving activity, an adult male walrus (no. 4344) that rested on Sando Island on 24 July 2001 was immobilised with etorphine HCl reversed with diprenorphine HCl (Born and Knutsen 1990a; Griffiths et al. 1993). During immobilisation a ST-10 satellite-linked radio transmitter (Wildlife Computers, Seattle, USA) was fitted to one of its tusks according to methods described in Born and Knutsen (1992) and Gjertz et al. (2001). In addition to providing data on location, the ST-10 transmitter was able to collect diving data to a depth of 500 m with a resolution of 2 m. Information on haul-out and at-sea time was collected via "timelines" (TIM) that stored data on the status of the salt water switch (SWS) (i.e. dry vs wet) of the transmitter in the course of 24 h in 20-min increments (Born et al. 2002). Blocks of 24-h timelines were transmitted every 40 transmissions.

Information on diving activity was summarised and transmitted per 6-h time blocks. The diving-depth data were transmitted as a histogram with each dive registered according to its deepest point. The duration of the dives was also transmitted as histograms. Dives had to be deeper than 4 m to be registered as dives. The histogram depth intervals were (m): 0–6, 6–12, 12–18, 18–24, 24–30, 30–36, 36–42, 42–48, 48–54, 54–60, 60–90, 90–120, 120–150, <150 m. The histogram duration intervals were: <1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, 9–10, 10–12, 12–15, 15–20, >20 min. The "time-at-depth" histogram intervals were (m): 0 (meaning that the first bin summarised the cumulative time the SWS had been dry; i.e. the transmitter had been dry at the surface), 0–6, 6–12, 12–18, 18–24, 24–30, 30–36, 36–42, 42–48, 48–54, 54–60, 60–90, 90–120, <120 m. In addition, the transmitter gave the depth of the deepest dive of the day. The transmitter was programmed for continuous sampling and transmission with a repetition rate of 45 s at sea and 90 s on land.

Total body mass (kg) of walruses can be estimated reliably from a combination of standard body length (SL, cm) and axillary girth (AG, cm) (equation 6 in Knutsen and Born 1994). TBM of three adult male walruses (age, years/TBM, kg: 12/839, 15/953 and 31/1629) that were weighed in Northeast Greenland in 2000 and 2001 (E.W. Born and M. Acquarone, unpublished data) were added to data in Knutsen and Born (1994) to establish an improved predictor of TBM. This predictor has the form: $\text{TBM (kg)} = C*\text{SL}^{A}*\text{AG}^{B}$; where C is a constant derived from the data ($n=15$). $\text{TBM} = e^{-8.81314}*\text{SL}^{1.03067}*\text{AG}^{1.74732}$; [SE $(C)=0.36873$; SE $(A)=0.30819$; SE $(B)=0.27354$]; $r^2=0.99$. Walrus 4344, which is the "type animal" of this study, had an SL of 292 cm and an AG of 319 cm. Accordingly, his estimated TBM was ca. 1,226 kg.

Results

Visual observations of diving activity

Visual observations were made in order to establish whether the feeding behaviour of the study animals was affected by the presence of the rubber dinghy and the scuba diver. On ten occasions, the diving and surfacing behaviour of foraging walruses up to a distance of about 1,200 m from the coast at Daneborg was recorded before, during and after underwater observations. These observations were made on four different days between 31 July and 7 August and lasted 30 min to 2 h 55 min. No significant difference was detected in duration of dives or at-surface time under undisturbed and "disturbed or diver-present" conditions, respectively ($P>0.05$; Mann-Whitney U-tests (surface and dive time), $n=104$, and two-way ANOVA (dives) with factors "disturbance" and "individual", $n=115$).

On these 10 occasions, a dive averaged 6.7 min (SD = 1.6; range: 1.9–12.1 min; $n=115$) with at-surface intervals of 1.0 min (SD = 0.5; range: 0.1–4.0 min; $n=104$). Sixty-nine percent of the dives lasted between 5 and 7 min. The dives were made in waters at between 8 and 34 m depth.

Hence, these observations indicated: (1) that the foraging behaviour of the walruses was not influenced by the presence of researchers above and under water, and (2) that walruses were submerged for about 87% of the time when foraging at water depths of less than ca. 32 m.

Number of bivalves consumed per dive

The ten dives that were studied under water were made between 1000 and 2200 hours on four different days by six different walruses judging from body and tusk size. During the 10 dives that were made within 200–350 m from the coast at an average depth of 9.7 m (SD = 3.7; range: 5.8–15.9 m), the walruses were submerged for an average of 5.8 min (SD = 0.7; range: 5–7 min).

Shells of *M. truncata*, *H. arctica* and *S. groenlandicus* were collected at the ten distinct patches resulting from the dives (Fig. 2B). On average, 53.2 bivalves had been consumed per dive of which ca. 72% were *M. truncata*, ca. 21% *H. arctica* and ca. 7% *S. groenlandicus* (Table 1). Interpreted from the size of the empty shells, the walruses obtained an average of 149.0 g shell-free dry matter during each dive (Table 1). *M. truncata* constituted 81.6% of the DM ingested per dive, *H. arctica* 3.7% and *S. groenlandicus* 14.7% (Table 1). An estimated 583 g wet weight bivalve biomass was ingested per dive (Table 1).

When adjusting for differences in energy contents and proportions of the three bivalve species in the individual dives, a walrus obtained an average of about 2,576 kJ during each dive (Table 2).

Activity during a foraging excursion

During late July and August, walrus 4344 made several foraging excursions from Sando Island, one of which coincided with the period in which individual dives were studied at Daneborg. Between 2 August 2300 hours and 6 August 0100 hours, 4344 went on a 74-h foraging trip

Table 1 Number of bivalves consumed during ten walrus dives that were studied under water in Young Sound (NE Greenland) between 31 July and 7 August 2001, with information on dry matter, energy contents and wet weight biomass of the ingesta (*SF* shell free; *DM* dry matter)

	All bivalve species combined			By species								
				Mya			*Hiatella*			*Serripes*		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
No. of bivalves per dive	53.2	5.2	34–89	38.2	4.6	20–51	11.0	2.1	0–20	4.0	3.9	
Shell free dry matter obtained per dive (g)	149.0	18.9	62.4–253.1									
Average DM obtained per dive by species (%)				81.6	4.61	56.7–100	3.7	0.94	0–10.1	14.7	4.61	0–41.4
% Dry matter of SF wet weight				24.3	0.77	19.5–27.2	36.9	2.16	26.1–55.1	30.6		
Energy (kJ/g DM)				17.4			15.4			17.0		
SF wet weight biomass consumed per feeding dive (g)	583	70.8	242 1000									

Table 2 Estimate of energy obtained per walrus feeding dive and estimates of energy consumed during a feeding cycle of a 1,226-kg male walrus in Young Sound (NE Greenland) between 2 and 6 August 2001 (*SF* shell free; *TBM* total body mass)

	Mean	SE	Range	*n*
Energy obtained per dive (kJ)	2575.5	325.2	1071.8 4377.2	10 dives
Total energy consumed during 412 foraging dives (kJ)	$1061.110*10^3$ (95% CI: $758.068*10^3$–$1364.152*10^3$)			
Total energy consumed per 24 h (kJ) during the 74 h "at-sea" period	$344.144*10^3$ (=82,331 kcal) (95% CI: $245.860*10^3$–$442.428*10^3$)			
	During 74 h at sea		During entire 97-h feeding cycle[a]	
Total energy consumed per 24 h (kJ per kg per day)	281 (=67 kcal) (95% CI: 200–360)		214 (=51 kcal) (95% CI: 153–275)	
Total amount of SF wet weight consumed per day (kg)	74 (95% CI: 54 95)		57 (95% CI: 41 72)	
Daily w.w. ingesta (kg) as % of TBM of the walrus	6.0 (95% CI: 4.4–7.8)		4.7 (95% CI: 3.3–5.9)	

[a] Included 74 at-sea and a subsequent on-land period of 23 h

to the Daneborg area and further east after having rested on Sando Island for about 37 h (Fig. 1). After its return to the island, the walrus hauled-out for another 23 h.

During the foraging trip, the maximum diving depth per day averaged 22.4 m (SD = 6.1, range: 16–32 m, $n = 5$ days). Overall, about 33.5% of all dives ($n_{total} = 775$) were between 0 and 6 m and the remainder between 6 and 32 m (64.5% of the dives were between 6 and 18 m, Fig. 3A). The distribution of duration of individual dives was bimodal, with 23.0% of the dives lasting less than 1 min and with a marked peak between 3 and

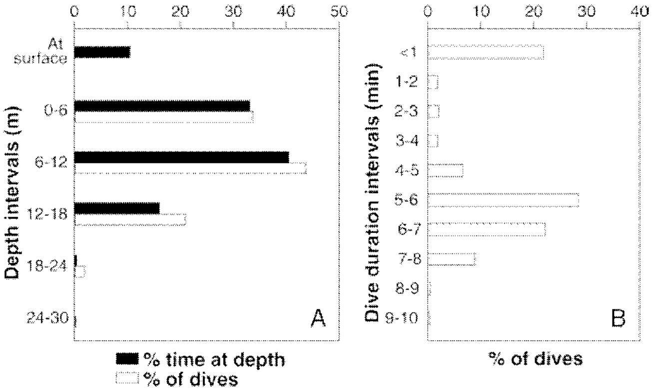

Fig. 3A, B Diving activity of an approximately 1,200-kg adult male walrus in Young Sound (NE Greenland) between 2 and 6 August 2001. A satellite-linked radio transmitter attached to one of the animal's tusks monitored its diving activity during a 74-h feeding trip. **A** Percentage of time spent at different depths ($n = 5,040$ min monitored, *black bars*) and percentage of dives ($n = 775$, *grey bars*) in different depth intervals during the trip. **B** Distribution of duration of individual dives ($n = 775$) made during the trip

9 min (71.7% of the dives) (Fig. 3B). When the walrus was in the feeding areas, the mean duration of dives lasting more than 1 min was 5.9 min (SD = 1.2; $n = 488$; 3 dives of 20 min or more—likely artefacts—were omitted from the analysis), which was not significantly different from the duration of the dives studied under water at Daneborg ($P > 0.05$).

During the foraging excursion, 43.3% of the time was spent at depths between 0 and 6 m, and about 10.3% of this time was spent at the surface; 56.7% of the time was spent between 6 and 32 m (Fig. 3A). A total of 515 dives went below 6 m. Of these dives, 80% ($n = 412$) lasted between 5 and 7 min. Dives between 5 and 7 min duration are typical of foraging (Wiig et al. 1992; Gjertz et al. 2001). It was therefore assumed that during all dives of this duration, animal 4344 went to the sea floor in successful search for food.

Estimates of food and energy intake during a foraging trip

To obtain an estimate of food and energy intake during a typical foraging excursion, the information on bivalve biomass and energy per dive (Tables 1, 2) was combined with the data on activity of our study animal during its feeding trip between 2 and 6 August.

Assuming that all 412 dives lasting between 5 and 7 min resulted in the intake of an average of 53.2 bivalves, the walrus ingested a total of 21,900 (95% CI: 16,300–27,500) bivalves during the trip, corresponding to a total of $1061.110*10^3$ kJ (or 281 kJ per kg body mass per day) (Table 2). Using the "dry-matter:wet biomass" ratios for bivalves in this study (Table 1), this is equal to ingestion of about 74 kg shell-free wet biomass of bivalve tissues per day, or about 6.0% of the total body mass of walrus 4344 (Table 2).

However, estimates of daily gross energy or food intake must also include periods of inactivity or rest. When the 23-h resting period that followed the trip at sea was included, the values for energy and food intake during the entire 97-h foraging cycle was: gross energy intake, 214 kJ per kg body mass per day; gross wet weight biomass ingested, 57 kg per day; gross food intake as percentage of TBM, 4.7 per day (Table 2).

Discussion

Diving activity

The observations at Daneborg of walrus diving activity and the information obtained from satellite-telemetry were typical of foraging walruses. Born and Knutsen (1997) observed that walruses were submerged for about 81% of the time during foraging and slow swimming and that the mean duration of a foraging dive was around 5 min. Fay (1982) reported that feeding walruses were submerged for 86% of the time (range: 83–89%). The average dive duration of a walrus fitted with a TDR was 5.2 min and 24% of the time was spent in the upper 2 m of the water column (Wiig et al. 1992). Gjertz et al. (2001) found an average duration of 6.0 min for foraging dives in walruses equipped with TDRs.

Types of bivalves eaten and estimates of the numbers ingested

The shells collected after each foraging dive represented three bivalve species that are known to make up the bulk of walrus diet. Fay et al. (1984) found that these bivalves constituted 87–98% of the number of consumed invertebrates and 54–80% of the volume of the ingesta. Vibe (1950) found them to be very abundant in stomachs of walruses in Northwest Greenland, with *M. truncata* numbering 1,170 (37%) and *H. arctica* 1,881 (60%) in a single stomach containing a total of 3,137 invertebrate food items.

In the present study, the estimate of the number of bivalves consumed during a typical feeding dive was about 53, corresponding to an ingestion rate of about 9 per minute dive (8 per minute dive if the at-surface time of a dive cycle is included). Oliver et al. (1983) were able to quantify records of walrus predation at six sites at the sea floor in Alaska. Walruses had foraged at these sites for at least 1 week, and probably several weeks, before the divers studied the records (i.e. empty shells and foraging pits and furrows). Based on a single feeding event that left signs of 19 *M. truncata* and 15 *S. groenlandicus* having been eaten, Oliver et al. (1983) estimated that walruses may consume more than 6 bivalves per minute. Based on this information and data on aerobic diving capacity of walruses, Kastelein et al. (2000) deduced that free-living walruses consume 40–60 bivalves per dive. Our findings agree with his estimate. One may speculate whether shells left on the sea floor from earlier dives were inadvertently included in the estimates of number of bivalves consumed per dive in the present study. However, each recent foraging site could be distinguished as a patch 2–3 m wide and relatively light in colour in the sandy bottom substrate. Furthermore, the shells left from the most recent foraging dive still had some soft parts attached. We therefore believe that the foraging rates in the present study are valid.

Amphipods, gastropods and other scavengers are abundant in the area (Sejr et al. 2000). Large numbers of mobile amphipods rapidly invaded the feeding pit and covered all the freshly eaten shells within minutes, followed by gastropods and later echinoderms. Most soft remains within the shells were devoured within 1–2 h as also experimentally documented by Gee and Warwick (1985). Several attempts other than the ten successful ones were made at diving with walruses and identifying the remnants from a feeding dive. However, in some cases it was not possible to identify a patch representing a single foraging dive because the walrus used the same patch during several consecutive dives. Hence, the ten dives included in this study represent occasions where it is beyond doubt that shells from only one recent dive were collected and identified.

Other studies (Vibe 1950; Fay et al. 1984; Welch and Martin-Bergmann 1990) have suggested that walruses mainly eat the "exposed" parts of bivalves (feet and siphons). However, in the present study only 2–3% of the soft parts was left on the shells of recently predated bivalves and the walruses clearly ingested almost all parts of the bivalves. This is in accordance with Sheffield et al. (2001), who deduced from laboratory experiments that walruses probably consume all the soft parts of their bivalve prey.

Species that constitute the most important portion of the walrus diet were included in the present study. However, it cannot be precluded that walruses in Young Sound also feed on bivalves other than the three species considered in this study. Although the small (<3 cm) bivalve *Astarte* spp., which has been reported to be walrus food (cf. Fay 1982), is very abundant (200–400 individuals/m^2) in the study area at depths <20 m (Sejr et al. 2000), open shells of this bivalve were not found at the walrus feeding patches. Numerous undamaged *Astarte* individuals lay scattered on the sea floor where a walrus had been feeding, so if the walruses *did* eat *Astarte* spp. as well, they must have swallowed them whole. However, no evidence was seen of shells in the walrus faeces at the haul-out on Sando Island. Sea cucumbers, gastropods and polychaetes have also been reported to be walrus prey (e.g. Fay 1982). These species are present in the fjord (Sejr et al. 2000) and it is likely that the walruses sometimes also consume them. However, the present study did not allow quantification of the role of this prey. Although polychaetes are abundant, very few large species are found (Sejr et al. 2000), and compared to the weight of the bivalves ingested, they must be inferred to play a minor role. Gastropods are relatively rare and are only found in abundance as scavengers at the walrus feeding pits. Although Born et al. (1997) reported an observation of opercula of *Buccinum* sp. in walrus faeces on Sando Island, no evidence was found in the present study in walrus faeces or in the feeding pits that gastropods are ingested to any extent. Sea cucumbers are mainly found at depths of 40–50 m. (Sejr et al. 2000), which is well below the preferred feeding depths of walruses observed in this study. Furthermore, historical observations of the diet of walruses feeding in the vicinity of Young Sound indicate that *M. truncata* and *Hiatella* sp. were principal food items (Peters 1874; Payer 1877a, 1877b). Hence the estimates of the present study of walrus ingestion rates inferred from the bivalves studied are likely to be representative.

Activity during a single foraging excursion

In order to calculate the food intake of free-living walruses, one feeding trip of an adult male walrus that coincided with the shore-based and underwater observations at the foraging ground was selected. However, the overall activity of walrus 4344 during the study period was typical of walruses foraging at inshore or near-shore mollusc banks.

The average duration of the foraging trip and the rests at the terrestrial haul-out prior to and after the excursion were quite similar to those reported by Born and Knutsen (1997) for male walruses at another haul-out in Northeast Greenland in August.

Walrus 4344 dived to 32 m with an average daily maximum depth of about 22 m, which is similar to mean maximum depths during inshore foraging of walruses equipped with TDRs at Svalbard (Wiig et al. 1992).

About 53% of all dives made by walrus 4344 went below 6 m and were thought to be associated with feeding. At Svalbard, about 40% of all dives of walruses equipped with TDRs were thought to be true foraging dives (Gjertz et al. 2001). Jay et al. (2001) found that Alaskan walruses fitted with TDRs used 46% of the trip making dives of about 7 min in duration likely associated with benthic foraging.

As witnessed by the stomach contents, walruses can take between 3,100 (Vibe 1950) and 6,400 prey items (Fay et al. 1984) in one meal. The predation rate in the present study (8–9 bivalves per minute dive) indicates that a walrus may be engaged in feeding for up to about 13–14 out of 24 h. Information on diving activity obtained from satellite-telemetry in Northeast Greenland indicated that walruses can be engaged in diving for about 17 h of a 24-h period (Born and Knutsen 1990b).

Aerobic dive limit (ADL) of walruses is around 10.5 min (Wiig et al. 1992). Less than 0.8% of the dives of our study animal exceeded 10 min (some 20+-min-long dives were thought to be artefacts) supporting the notion that walruses, by not exceeding their ADL, forage in an energetically efficient way.

Estimates of food and energy intake

The estimate of daily food intake of free-living walruses in the present study is higher than daily food and energy intake reported by Fisher et al. (1992) and Kastelein et al. (2000) for captive Pacific walruses in the Netherlands. Based on a study of captive walruses for 2 months, Fisher et al. (1992) estimated that a 1,200-kg non-pregnant, non-lactating adult walrus required 109 kJ per kg per day. The highest energy intake observed was 193 kJ per kg per day in a 550-kg female. However, all except one of the study animals lost weight during the study carried out by Fisher et al. (1992), both when being fed a daily diet of comparatively energy-rich fish (26.6 kJ per g DM) and a diet of clams (*Spisula* sp.; energy contents, 20.4 per g DM). Daily gross food intake varied between 1.3 and 1.8% of TBM. Apparently, the walruses in the study carried out by Fisher et al. (1992) received too little food. Kastelein et al. (2000) studied the food intake of captive walruses during several annual cycles. In their study, two captive walruses between 1,000 and 1,200 kg (1 M and 1 F) that were fed mainly fish consumed an average of 2.4% of TBM per day (range: 1.8–3.1%, inferred from Fig. 9 in Kastelein et al. 2000), which apparently was enough for maintenance.

We did not determine the energy content of *Serripes* sp., of which mainly the foot is taken by walrus (Vibe 1950). Instead, an average of the energy contents (17.0 kJ per g DM) determined in the two other principal food items was used. According to Brawn et al. (1968), the energy content of *Clinocardium* sp.—another principal walrus food of which mainly the feet are taken—is 18.6 kJ per g DM. However, using this estimate for content of energy in *Serripes* in the present study will only raise the estimate of energy consumed in a single dive by about 1%. Hence, adjusting the estimate of energy in *Serripes* only has a negligible effect on the estimate of the present study.

It seems that the energy content of the bivalves consumed in the present study was generally lower than that in the food items used by Fisher et al. (1992) where a 1,350-kg male walrus that was fed clams lost weight. The fact that the energy content of the bivalves in Northeast Greenland was 15–25% lower than that of the food items used in the study by Fisher and co-workers would in itself imply the need for a higher daily gross food intake. However, the studies by Fisher et al. (1992) and Kastelein et al. (2000) were carried out on captive walruses at lower latitudes, where relatively high ambient temperatures prevail all year round. Furthermore, captive walruses are probably less active than free-living ones, and are usually fed a diet containing comparatively energy-rich fish. However, the food intake of walruses varies with season (Kastelein et al. 2000). To our knowledge, no one has attempted to estimate the energy consumption of free-living walruses during seasons other than summer. We therefore suggest that the estimates of food intake in the present study are tentatively used only for the open-water season.

The estimates of daily gross energy and food intake in the present study are close to those predicted for wild-living walruses. Genrich (1984) *fide* Fisher et al. (1992) calculated the gross energy intake of a walrus to be 936–1,157 kJ per kg$^{0.75}$ per day (i.e. 160–197 kJ per kg per day) based on an estimated TBM of 1,050 kg for wild walruses. Nine young captive walruses (TBM: 250–750 kg) that were mainly fed a diet of fish and *Mya* sp. consumed 4.2–6.2% of TBM per day (mean: 5.2%, SD = 0.1) (Fay 1982). At that rate, a free-living average adult male (TBM: 1,200 kg) would consume 50–74 kg per day (Fay 1982). In three captive male walruses between 642 and 750 kg, the daily gross energy intake ranged between 289 and 383 kcal per kg$^{0.75}$ (Fay 1982), equivalent to 220–312 kJ per kg per day. Fay (1982: Table 25) estimated that a free-living walrus between 1,000 and 1,200 kg would consume between about 42 and about 92 kg of molluscs per day, corresponding to between 4.2 and 7.6% of TBM per day.

Nagy et al. (1999) presented generalised allometric equations for calculation of Field Metabolic Rate (FMR) based on studies of energy expenditure that involved the use of doubly labelled water in free-living animals. According to Nagy et al. (1999), the daily FMR (kJ) in taxonomically defined carnivores can be estimated from $\mathrm{FMR} = 1.67\ \mathrm{TBM}\ (\mathrm{g})^{0.869}$, and from $\mathrm{FMR} = 2.23\ \mathrm{TBM}\ (\mathrm{g})^{0.850}$ in ecologically defined carnivores. These equations predict an FMR of a 1,226-kg walrus of 266 kJ per kg per day or 272 kJ per kg per day. The estimate of daily gross energy intake made in the present study is not directly equivalent to that of an estimate of FMR. However, the fact that the two estimates in the case of the 1,226-kg walrus are in the same order of magnitude indicates that the estimates of daily food intake in a free-living walrus made in the present study and in Fay (1982) are reasonable. Hence, the estimates of daily consumption rate (i.e. 5.7% of walrus TBM) used in calculations by Welch et al. (1992) and Weslawski et al. (2000) of walrus predation in the wild were at the high end of the range of uncertainty associated with our estimate of walrus food intake.

The impact of the walruses on the bivalve community

In Northeast Greenland, the inshore mollusc banks are covered by a solid layer of ice except during the short open-water period, and are therefore inaccessible to walruses most of the year (Born et al. 1997). The walruses are forced to winter offshore, sometimes over relatively deep waters (> 100–200 m) (Born and Knutsen 1992) where they are unable to feed effectively (Fay 1982; Fay and Burns 1988). However, during the summer period, they have access to plentiful inshore food resources. A high standing stock of bivalves is present in the study area (Sejr et al. 2000, 2002), which is representative of other inshore ice-covered Arctic areas (Berthelsen 1937; Vibe 1939, 1950; Ockelmann 1958; Thomson et al. 1986; Grebmeier et al. 1989; Welch et al. 1992). Given the estimate of daily gross food intake of a walrus made in the present study, the standing stock of *H. arctica* and *M. truncata* in the outer Young Sound represents a food supply corresponding to about 153,000 walrus feeding days. Young Sound is free of ice only 80–90 days per year, and the maximum population that may feed in the area counts about 50 animals (usually much fewer are seen in the area; Born et al. 1997). This means that the walrus population is able to consume < 3% of the standing stock of *H. arctica* and *M. truncata*, or less than half of the annual somatic bivalve production (M. Sejr, unpublished work). The standing stocks and productivity in Young Sound of other walrus food items, for example *S. groenlandicus*, have not been determined. If these are also considered, the inshore bivalve banks in Young Sound represent a rich food source, which is not yet fully exploited by walruses.

Based on observations of walrus distribution in the Beaufort and Chukchi Seas in 1998, Kelly (2001) suggested that a decreased extent of summer sea ice may negatively impact the ability of Pacific walruses to obtain food. During the summer of 1998, the sea ice in these areas had retreated unusually far to the north. Consequently, substantial portions of the ice edge—where females and young occurred—had receded north of the continental shelf where the water was too deep for walruses to feed.

The scenario described by Kelly (2001) may apply to the situation for Pacific walrus in the Bering Strait region, but not necessarily to groups of Atlantic walrus in areas where they feed inshore during summer (i.e. in eastern Greenland, the Canadian High Arctic and the Svalbard-Franz Joseph Land region). A decrease in Arctic sea ice and consequent increase of the open-water period will increase the time in which Atlantic walruses in such areas have access to the food-rich coastal areas.

Previous studies indicate that a prolonged open-water period will increase the marine primary production in the area (Rysgaard et al. 1999). Improved food availability will stimulate bivalve growth and production and help balance the potential increase in walrus predation. Since walruses use the inshore period to feed intensively, a future prolonging of the open-water period can be expected to improve foraging conditions for walruses and thereby enhance the population of Atlantic walruses. This may be the case, not only in eastern Greenland, but also in other areas of the Arctic with similar walrus habitats.

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Paper VI: An estimation of walrus (*Odobenus rosmarus*) predation on bivalves in the Young Sound area (NE Greenland)

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Abstract

The total consumption of bivalve prey by walruses (*Odobenus rosmarus*) in the important inshore summer feeding area Young Sound (about 74°N) in Northeast Greenland was estimated. To determine relative area use, the movement and activity of three adult male walruses with satellite transmitters were studied during the open water season in 1999 and 2001. Because one of the animals was tracked during both years the study covered a total of four "walrus seasons". Overall, the animals used ca. 30% of the time in the water in the inshore study area in Young Sound. The remainder of time was used along the coast north and south of Young Sound and offshore in the Greenland Sea. Information on the number of walruses (n=40), occupancy in the study area, and estimates obtained from satellite telemetry on the number of daily feeding dives (118-181/24 hr. at sea), were used to calculate the amount of bivalve food consumed by the walruses during a total of 1080 "walrus feeding days" inshore in Young Sound. Depending on the applied estimator of number of feeding dives, the estimated consumption by walruses of shell-free (SF) bivalve wet weight (WW) during the open water period range between 74 and 114 tons. Based on estimates of mean total body mass (TBM: 976 kg) of walruses using the area and daily *per capita* gross food intake, the corresponding estimate of consumption by walruses is 63 tons SF WW. Daily feeding rates in walruses (6-7% of TBM) indicate that the estimates of 63 tons and 74 tons are the most plausible. Hence, during the open water season walruses consume between ca. 1.9 and ca. 2.2 g/m^2 SF bivalve WW at < 40 m depth in Young Sound. These estimates of predation amount to 1.2 - 1.3% of the standing biomass of the important walrus prey *Mya* sp. and *Hiatella* sp. only, and 19-28% of the annual production of these bivalves.

Introduction

Major climatic changes in the Arctic due to global warming may affect walruses (*Odobenus rosmarus*) in various ways. Kelly (2001) suggested that a decreased extent of summer sea-ice might negatively impact the ability of Pacific walruses (*O. r. divergens*) to obtain food in the Beaufort and Chukchi Seas. Born et al. (2003) hypothesized that in areas such as eastern Greenland, Svalbard and the Canadian High Arctic archipelago where Atlantic walruses (*O. r. rosmarus*) feed intensively inshore, a reduced ice cover may positively affect the walruses by allowing them access to their feeding areas for a longer period of time. Furthermore, an extension of the duration of the open water period may enhance marine productivity in general (Rysgaard et al. 1999).

In the eastern Atlantic Arctic, including the East Greenland and Svalbard areas, the ice cover has decreased during the last 2-4 decades both in thickness and extent due to global warming (Rothrock et al. 1999, Parkinson 1992, 2000, Comiso 2002). Temperatures have increased in the East Greenland – Svalbard area since the 1960s (Førland et al. 2002, Hanssen-Bauer 2002). Furthermore, a continued dramatic temperature increase and associated reduction in ice cover in the East Greenland area are predicted during this century (Rysgaard et al. 2004).

To evaluate the effects of the reduced ice cover on the Arctic marine ecosystems and their productivity, a multi-disciplinary study CAMP (Changes in the Arctic Marine Production) was initiated in 1995 (e.g. Rysgaard et al. 2000). The focal site of this study is Young Sound (ca. 74°15′N) in Northeast Greenland where a small group of walruses feed intensively on the inshore mollusk banks during summer. Because walruses are a conspicuous and important component of this ecosystem it was necessary to quantify their trophic role.

The stenophagous walruses are an important component of many High Arctic marine ecosystems where they predate on the benthic invertebrate fauna in coastal waters (e.g. Vibe 1950, Fay 1982, Oliver et al. 1983). Although walruses may feed on a variety of bottom-dwelling invertebrates, only a few bivalve species, usually *Mya* sp., *Hiatella* sp. and *Serripes* sp., make up the bulk of their diet (Vibe 1950, Fay 1982, Fay et al. 1984, Sheffield et al. 2001). The Young Sound study area has a rich benthic infauna that includes abundant quantities of these three walrus food items (Sejr et al. 2000, 2002, Sejr 2002, Born et al. 2003).

A small group of walruses, genetically distinct from the neighboring Svalbard and West Greenland groups (Andersen et al. 1998, Born et al. 2001) lives all year round in eastern Greenland where they are mainly distributed north of about 72°N (Born et al. 1997). Apparently, the walrus sub-population in eastern Greenland was on the verge of extinction in the 1950s due to over-exploitation by European whalers and sealers. However, since its protection in 1956 the East Greenland sub-population of walruses has shown signs of a slow increase (Born et. al. 1995, 1997).

Two main areas are known where East Greenland male walruses concentrate to feed during summer: The Dove Bay area (76-77°N) and the Young Sound area (Figure 14). The only two regularly used terrestrial haulout sites in Greenland are found in these areas: Lille Snenæs in Dove Bay, and the island of Sandøen in Young Sound. Females are distributed along the coast farther north (Born et al. 1997). In recent years up to about 50 adult male walruses have been observed hauling out simultaneously on Sandøen (*ibid.*, Born & Berg 1999).

Based on direct underwater observations of feeding walruses and satellite-telemetered information on diving activity, Born et al. (2003) estimated the amount of food ingested per single feeding dive and during a typical feeding trip.

In the present study we estimate the total predation pressure exerted by walruses on the bivalve population of the Young Sound area during the open water season. This is done by combining information on (1) movement and diving activity in Young Sound of individual walruses equipped with satellite-linked transmitters (this study), with (2) information on food ingested per dive and daily *per capita* feeding rate (Born et al. 2003), and (3) an estimate of number of walruses frequenting the area during the open water period (Born et al. 1997, Born & Berg 1999, L.W. Andersen & Born, unpubl. data).

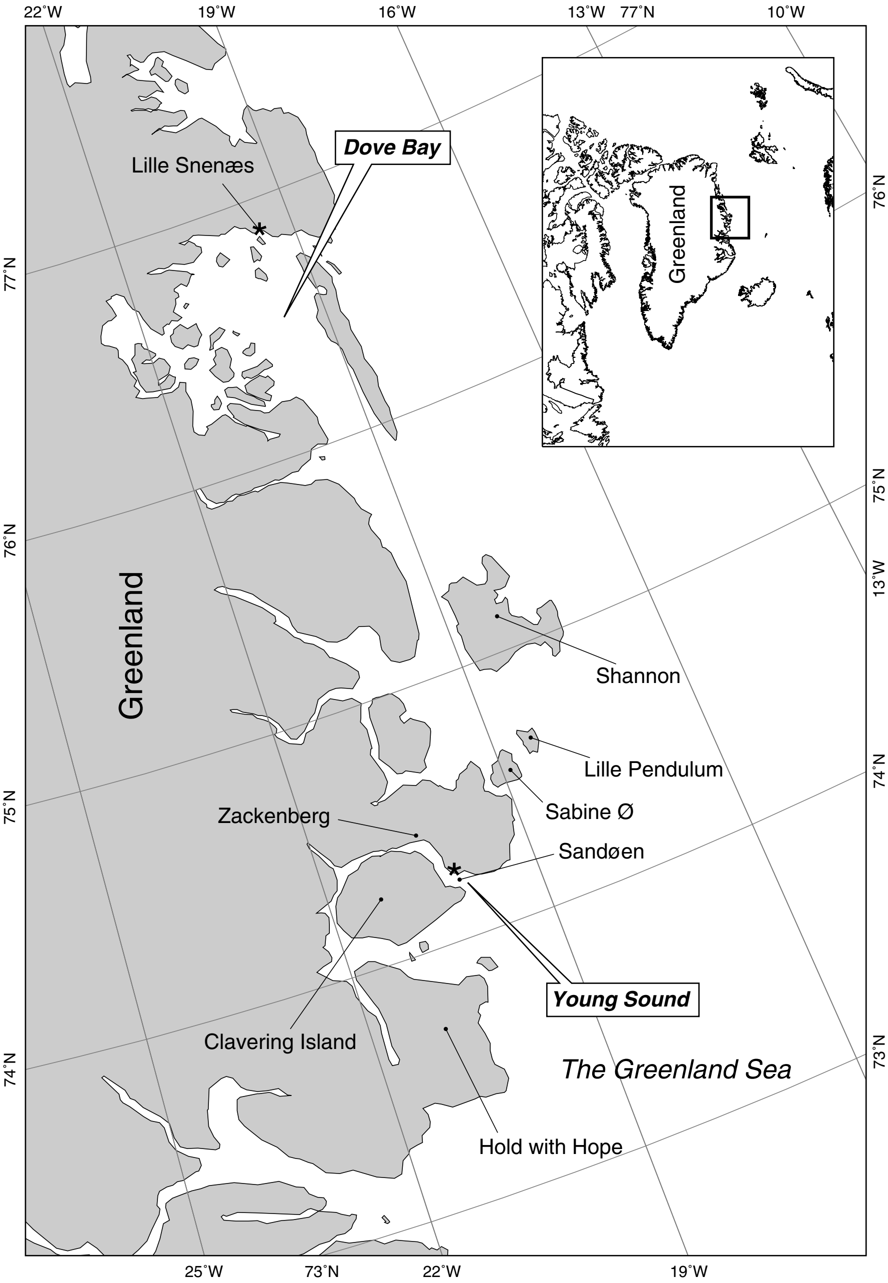


Figure 14 Map of the study area in northeastern Greenland. The Sandøen and Lille Snenæs haulouts are indicated (*).

Materials and methods

The study animals

During August 1999 and July 2001 three individual adult male Atlantic walruses that hauled out among other male walruses on the beach of Sandøen (74°15′30″N, 20°18′00″W) in Young Sound (NE Greenland) were immobilized with etorphine (Born & Knutsen 1990a, Griffiths et al. 1993); Table 16. Estimates of total body mass (TBM) of these animals were obtained from equations on TBM versus standard body length and girth in Knutsen and Born (1994); Table 16. Their approximate age was estimated from a "tusk circumference-at-age" relationship obtained from walruses sampled in NW Greenland

(Circumference in cm at basis = 3.0 (SE: 0.94) + 20.5 (SE: 2.57) * (1-exp[-0.068(SE:0.02) *age in years])).

Animal 6481 was tracked in 1999 and 2001 (4344); Table 16. Because its movements and diving activity differed in the two seasons it is treated as two different "cases" in the analyses of activity. Hence, a total of four, individual, "walrus seasons" were included in the study.

Tracking of movement

During immobilization, a satellite-linked radio transmitter was attached to one of the animal's tusks as described in Born and Knutsen (1992) and Gjertz et al. (2001). Two different types of satellite transmitters were used. In 1999 and 2001, respectively, a ST-10 transmitter was put on an individual that was tracked in both years. In 2001, a SPOT-2 was fitted to the tusks of two other walruses (Table 16). Wildlife Computers (Seattle, USA) manufactured all transmitters.

All the transmitters were able to provide data on location, but their sampling protocols for collection of sensor data were different (*cf.* section "Activity data").

The GIS software ArcView 3.2a was used for mapping the movement of the walruses. For analyses of movement and area use (*cf.* Harris et al. 1990) all position data of all quality classes were run through the PC-SAS®ARGOS-filter that chooses the most plausible location between the ARGOS primary and alternate locations based on minimum distance from the previous chosen location irrespective of the class (V.5.0, D.Douglas USGS, Alaska Science Center, 100 Savikko Road, PO Box 240009, Douglas, AK 99824, USA, unpubl. method). The parameters used during filtering were: Maximum Redundancy was (Distance, rate, angle filter) = 5 km. Minimum movement rate = 10 km/h. Rate coefficient = 15.

Activity data

In the present study, the area for which feeding by walruses was quantified encompassed the areas in Young Sound west of Sandøen and north of 74°14´ N (i.e. on the northern coast of Clavering island due south of Sandøen). For each animal the approximate time spent inside (i.e. "total time spent inshore") and outside the study area during the open-water season was inferred from the satellite-telemetered locations. Time spent inshore for an animal was defined as fraction of days with locations in Young Sound west of Sandøen of all days monitored during the open water period.

The animals were tracked for different periods of time (Table 16) but their feeding activity was only described and quantified for the open water season (for periods monitored see Table 17).

The ST-10 transmitter used in 1999 was able to collect diving data to a depth of 250 m whereas that deployed in 2001 had a maximum depth range of 500 m (Table 16). Maximum dive depth of the SPOT-2 transmitters was not specified.

For the ST-10 units, information on haul-out time (duration of individual haul-outs and % of time hauled out) was collected via ”timelines”(TIM) that stored data on the status of the salt-water switch (SWS; i.e. dry versus wet) in the course of 24 hr. in 20 min increments (Born et al. 2002, 2003). Blocks of 24-h timelines were transmitted every 40 transmissions.

The SPOT-2 transmitters were not able to collect dive data or information on the activity of the SWS. For these transmitters, the haul-out time was inferred from temperature data and locations. These units transmitted temperature information summed in 6-hr blocks. The temperature histograms were stored in 14 user-defined intervals. For the present purpose, all 6-hr blocks in which the temperature was 4°C or higher were assumed to represent a period where the animal was hauled out and exposing the sensor to its own or another walrus’ body-heat or in air temperatures. Mean temperatures in Young Sound are below freezing 9 months of the year and only the months June to August have a positive mean air temperature of up to 4°C (Rysgaard et al. 2004). In case a 6-hr histogram both contained values below and above 4°C, it was assumed that the walrus was hauled out if ≥75% of the time was used at ≥4°C (only < 3% of all 6-hr blocks were categorized as representing a haul-out period based on this criterion). The reception during the same periods of good quality locations (location class 3 or 2; *cf.* Harris et al. 1990) was regarded as a confirmation of the fact that the animal was actually hauled out.

Percentage of time spent in water inshore was determined as ”total time spent in the study area minus percentage of time hauled out”.

The ST-10 transmitters sampled time and pressure (depth) every 10 sec. These data were stored in 6-hour blocks and then relayed to the satellite during the following 24 hours. Three types of dive data were used in the present study: (1) number of dives per time unit, (2) Duration of individual dives, (3) daily maximum dive depth (MDD), and (4) time at depths (TAD). Dive data were stored in 14 user-defined intervals that later were organized in the following intervals for analysis: 0-6 m, >6 m. For analyses of diving activity (i.e. number of dives to different depths), haul-out time was extracted from the dive data.

Heavy floes of multi-year ice come into Young Sound from the Greenland Sea during summer. Scouring of the sea floor by this ice and icebergs have resulted in relatively low densities of bivalve infauna < ca. 6 m depths along the shores (Sejr et al. 2000; Sejr 2004). It was therefore assumed that dives shallower than 6 m depth represented traveling and social activity whereas all dives deeper than 6 m depth were feeding dives.

Table 16 Identification code, date of instrumentation, type of satellite transmitter, estimate of total body mass (TBM) and approximate age of three different adult male walruses that were tracked in the Young Sound area (NE Greenland) in 1999 and 2001.

ID	Transmitter type	Output (Watt)	Depth range (m)	Date of instrumentation	Last location	TBM[2] (kg)	Age[3] (year)
6481	ST-10	0.25	250	23 Aug. 1999	21 Nov. 1999	950	24 (at least 13)
4344[1]	ST-10	0.25	500	24 Jul. 2001	4 Sep. 2001	1200	26 (at least 15)
11272	SPOT-2	0.50	-	27 Jul. 2001	14 Oct. 2001	1400	26 (at least 14)
6482	SPOT-2	0.50	-	28 Jul. 2001	24 Oct. 2001	1100	29[4]

1: Same animal that was tracked as 6481 in 1999

2: TBM = Total Body Mass estimated from body dimensions (Knutsen & Born 1994)

3: Age estimated from a "tusk circumference on age" relationship (cf. Materials and methods)

4: In 2002, hunters at the entrance to Scoresby Sound killed this animal and therefore molar teeth for estimation of age became available. Age was estimated from counting of growth layers groups in tooth cementum following the method of Mansfield (1958).

The number of feeding dives per 24 hr. was determined in two ways: (1) the number of dives exceeding 6 m was extracted from the ST-10 satellite transmitters and the number of dives below 6 m/24 hr. in water was calculated. In this analysis, which only included days spent inshore, all 6-hr blocks with no dives (= the animal was hauled out) were omitted; (2) the number of dives of between 5 and 7 min duration (i.e. typical feeding dives; Born et al. 2003 and references therein) were summed for all inshore days and the average number of 5-7 min long dives per 24 hr. at sea (“wet hr”) was calculated omitting 6-hr blocks where the animal had not dived (i.e. was hauled out).

Table 17 Estimates of haul-out time (%) for four adult male walruses when being inshore in the Young Sound area (NE Greenland) in 1999 and 2001.

ID	Period monitored	Total[1] hours monitored	Hours[2] inshore	% time spent inshore	% of total time spent in the water inshore[3]
6481	24 Aug.[4] - 2 Oct. 1999	960	419	43.7	33.0
4344	24 Jul. - 2 Aug. 2001[5]	228	228	100.0	34.4
11271	27 Jul. - 2 Oct. 2001	1620	357	22.0	10.9
6482	28 Jul. - 5 Oct. 2001	1668	948	56.8	44.9
All	All months, both years	4476	1952	43.6	29.5

1: Period until formation of ice cover in Young Sound

2: At Sandøen and west of this island

3: Haul-out time subtracted (cf. Table 18)

4: Day of instrumentation not included

5: Location received until 4 Sep. but after filtering last validated location was from 2 August 2001

Number, age composition and TBM of walruses

The group of walruses using the Young Sound study area during summer was assumed to number 40 (up to 50 individuals in some seasons). These estimates were based on (1) genetic identification using 11 nuclear markers (i.e. microsatellites) of 40 individuals among 81 biopsies taken from walruses at Sandøen during August

2002 (L.W. Andersen and E.W. Born, unpubl. data), and (2) maximum day counts of 47 in 1991 (Born et al. 1997) and 48 in 1994 (Born & Berg 1998). For calculation of food consumption, 40 walruses were considered as a best estimate of the average number of animals using the area.

By combining (1) the theoretical age composition with (2) estimates of age-specific TBM, an estimate of the average TBM of the walruses in Young Sound was obtained:

(1) Atlantic male walruses become physically and sexually mature when they are ca. 12 years old (Knutsen & Born 1994, Born 2003) when they also become fully recruited to the Inuit´s catch (Born et al. 1995). A representative age composition of the walrus population in East Greenland does not exist. Hence, the "recruited" part (ages ≥ 12 years) of an age-structured sample of 160 male walruses (ages: 12 to 29 years) that had been caught in NW Greenland (1989-1991; Born unpubl. data) was assumed to represent the age composition among adult walruses in Young Sound. The $\log_{10}$ of the number per age class in the NW Greenland sample was given by this linear regression: log-y = 2.499 – 0.086 * age ($r^2 = 0.889$). This relationship was used to establish the age composition in a group of 40 walruses that use Young Sound (Figure 15).

(2) Estimates of individual TBM of in the group of 40 walruses at Sandøen were obtained from Knutsen and Born (1994:eq. 7b); Figure 16.

Estimates of shell-free (SF) bivalve wet weight (WW) biomass and dry matter (DM) obtained during single feeding dives were obtained from Born et al. (2003).

The duration of the open water season (i.e. time from break up of the fast ice in spring until formation of fast ice in the fall), in which walruses have access to the inshore mollusk banks in Young Sound, was 76 d in 1999 and 108 d in 2001 (Rysgaard, in litt. 2003). For simplicity, an open-water period of 90 d is assumed in the calculations.

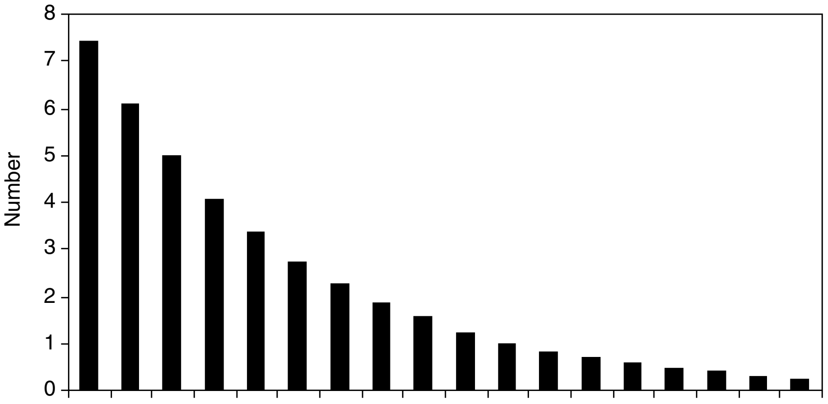


Figure 15 Age composition among 40 adult male walruses that use the Sandøen haulout in Young Sound during summer (cf. Materials and methods).

Data on the total area of suitable walrus feeding habitat in Young Sound between Sandøen and Zackenberg from the coast line to 40 m depth (Figure 14) were extracted from Rysgaard et al. (2003: Table 16).

Information on biomass and production of important bivalve prey in Young Sound was obtained from Sejr (2004).

Estimation of walrus consumption of bivalves in Young Sound

The total amount of bivalves consumed by the walruses in Young Sound during the open water season was estimated by two methods:

(1) Information was combined on (a) relative time in the Young Sound study area during four "walrus seasons" by three walruses that were tracked by use of satellite telemetry, (b) satellite-telemetered information on diving activity, (c) estimates of food ingested during single dives, (d) total number of walruses hauling out on Sandøen in Young Sound, and (e) total duration of the open water period.

(2) Information was combined on (a) the average TBM of walruses using Young Sound, (b) food consumed (6.0% of TBM/walrus/24 wet h, 95% CI: 4.2-7.5; Born et al. 2003), (c) total number of walruses in Young Sound, and (d) total duration of the open water period.

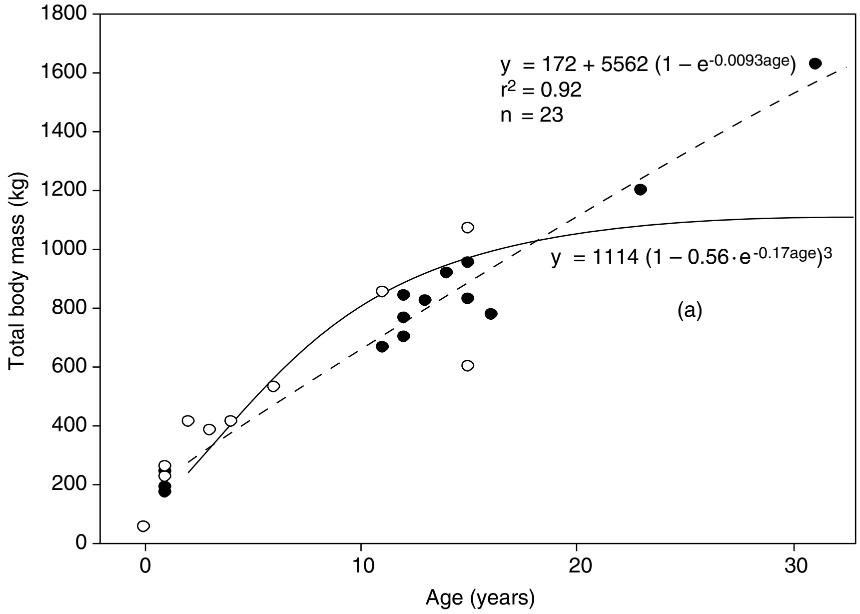


Figure 16 Total body mass (TBM) of male Atlantic walrus from Greenland (black circles, n=13) and Canada (open circles, n=10; extracted from Mansfield 1958: figure 13). The von Bertalanffy growth curve (a) of Knutsen and Born 1994 (figure 2, eq. 7b) (solid line), and a growth curve (b) established on the present data points (hatched line), are shown. TBM of three 0-1 year old female walruses (Born and Knutsen 1994) from NW Greenland were included to improve the predictive power of equation "b".

Results

The study area

The Young Sound study area is described in depth in Rysgaard et al. (2003) and Born et al. (2003). For the purpose of this study it is important to notice that a sill across the fjord by Sandøen divides Young Sound into an offshore and inshore area. Inshore, along the coast west of Sandøen up to Zackenberg (Figure 15) there is an abundance of shallow water banks rich in walrus food items (e.g. Sejr 2002, 2004). Further inshore (i.e. west of Zackenberg) the fjord is > 60 m. Walruses are not seen in this area and as the study animals did not enter this part of the fjord it is likely not good walrus feeding habitat. Hence, for the quantification of bivalve food consumed by walruses in Young Sound only the areas around and west of Sandøen (Figure 14) up to Zackenberg are considered. In this part of the fjord the area in which walruses forage down to 40 m depth is 33.2 km^2.

The study animals

The estimated TBM and individual age of the three adult male walruses that were tracked by use of satellite telemetry during 1999 and 2001 ranged between 950 and 1400 kg and ca. 24 and ca. 29 years, respectively (Table 16).

Movement and area use

Animal 6481 was tracked from instrumentation on 23 August on Sandøen until 21 November 1999. During this period it used Young Sound but also moved north and south along the coast (Figure 17). The reception of several high-quality locations (LC = 3) on the southwestern coast of Sabine Ø and from the southeastern coast of Clavering island indicated that 6481 also hauled out on land at these places. In 1999 this walrus spent about 44% of the time inshore in Young Sound either hauling out on Sandøen or in the water (Table 17).

The same individual was tracked in 2001 as 4344 from 24 July until 4 September (Table 16). However, after filtering of the locations its movement could only be reliably described until 2 August (Figure 18) until which date it remained inshore (Table 17).

Animal 11272 was tracked from 27 July until 14 October 2001 during which time it made excursions offshore in the Greenland Sea as well as north and south of Young Sound (Figure 19). Judged from the locations, 11272 spent about 22% of the time before formation of fast ice in the study area (Table 17).

Walrus 6482, which was tracked between 28 July and 24 October 2001 also made trips from Sandøen north to the Sabine Ø area and south to the southern coast of Clavering island (Figure 20). This animal used about 57% of the open water period inside the Young Sound study area (Table 17).

Overall, the locations indicated that the four walruses used a weighted average of about 44% of the time in the Young Sound study area (Table 17). When at sea in Young Sound, the locations indicated a clear preference for the areas in northern parts of the fjord where water depths are <40 m (Figure 17 and Figure 20).

Haul-out and diving activity

During the open water period the four walruses hauled out for between ca. 21 and ca. 66% of the time. Overall, haul-out time averaged 31.4% (Table 18). If individual haul-out time is subtracted, the walruses spent between about 11 and about 45% of the time in the water inside the study area (Table 17). Overall, the satellite telemetered information indicated that on average the walruses spent about one third (29.5%) of the open water season in the water in the Young Sound study area (Table 17).

Table 18 Estimates of haul-out time (%) for four adult male walruses when being inshore in the Young Sound area (NE Greenland) in 1999 and 2001.

ID	Period monitored	% of time hauled out	Total days monitored	No. of 6-hr blocks monitored
6481	25 Aug. - 29 Sep. 1999	24.3	16	-
4344	24 Jul. - 2 Aug. 2001	65.6	10	-
11271	1 Aug. - 23 Aug. 2001	50.5	26	97
6482	28 Jul. - 5 Oct. 2001	21.0	70	276
All	All months, both years	31.4	122	-

Data on diving activity during the open water season were only available for animal 6481/4344. For both years and all months combined, an average of ca. 32% of the "at sea" time was spent between 0 and 6 m depth (about 11% of this time was used at the surface; i.e. SWS dry), and the remainder of the time was used at depths below 6 m (Table 19). Less than 1% of the time was used at depths below ca. 40 m.

Table 19 Time (%) spent in different depth intervals (TAD) by an adult male walrus (same individual in both seasons) inshore in the Young Sound area (NE Greenland) in 1999 and 2001.

ID	Month	Year	% time at different depths			Days monitored	No. of 6-hr blocks
			At surface[1)]	0-6 m[2)]	>6 m		
6481	Aug.	1999	9.2	39.8	60.2	9	30
	Sep.	1999	11.8	29.8	70.2	29	88
	Oct.	1999	7.6	25.1	74.9	4	9
4344	Jul	2001	11.1	31.9	68.1	6	22
	Aug.	2001	15.8	37.2	62.8	2	2
	All	99+01	11.0	31.9	68.1	50	151

1: Time when the salt-water switch was dry

2: Includes time at surface.

About 80% of all dives inshore went to depths of 6 m and deeper. During the different months the animal made between 100 and 198 dives/24 wet hr. below 6 m, with an average of 181 dives/24 wet hr. (Table 20). Less than 1% of the dives went deeper than ca. 40 m.

The duration of a walrus feeding dive is usually 5-7 min (Born et al. 2003 and references therein). During the inshore period, about 61% of all dives of 6481/4344 lasted between 5 and 7 min (ca. 77% of all dives were between 4 and 8 min in duration), Table 21. When inshore, the animal made an average of about 118 dives of 5-7 min duration per 24 h. Less than 2% of the dives had a duration above 8 min.

On days when 6481/4344 was inshore, the daily maximum depth readings averaged 35.4 m (SD=24.6, range: 14 – 86 m, n = 7 days with maximum dive depth data) in 1999, and 26.5 m (SD=7.1, range: 20 – 36 m, n = 8) in 2001, which is in accordance with direct observations (Rysgaard and Ehlmé, pers. comm.) that walruses in Young Sound mainly feed on the shallow water bank along the shores. In none of the years did the maximum dive depths differ between inshore and offshore days (unpaired t-tests; $P > 0.05$). 6481/4344 made a maximum depth to 136 m on 30 August 1999 at 74°40'N and 18°34'W (i.e. outside Young Sound).

The number of walruses in Young Sound

The number of animals hauling out on Sandøen likely reflects the number using Young Sound and adjacent areas for feeding during summer. Mainly adult males haul out on Sandøen, and observations of females or immature individuals are very rare (Born et al. 1997, Born et al. 2000, unpubl. data).

Opportunistic and systematic observations (Born & Berg 1999, Born unpubl. data) of the number of walruses hauled out at Sandøen have been carried out since 1983. The daily maximum number of hauled out individuals ranged between 3 and 48. The highest numbers were recorded in 1991 (47) and 1994 (48) (Born et al. 1997, Born & Berg 1999). The maximum number seen on one occasion in late July and August 1998-2002 varied markedly (1998: 28, 1999:9, 2000: 22, 2001: 19, 2002: 19; Born & Berg 1999, Born et al. 2000, Acquarone et al. 2001, Born unpubl. data). In 1999 when the lowest number was observed, unusually much pack ice coming from the Greenland Sea occurred in Young Sound. Sometimes this ice blocked the beach at the walrus haulout probably precluding access to the haul out. However, during all seasons it was clear from observations of individually recognizable animals (*cf.* Born et al. 1997 for methods) that the number of walruses frequenting the haulout during August was higher than the highest number seen on any single occasion. This was confirmed in 2002 when the daily maximum count during the period late July – all August was 19 whereas genetic identification *post hoc* revealed that a total of 40 different animals used the haulout during the same period.

The average TBM in the walrus group using the area based on the theoretical age structure (Figure 16) and estimates of individual mass was 976 kg (range: ca. 890 kg at 12 years of age and ca. 1100 kg at 29 years). The total mass of the 40 walruses summed to ca. 39 tons. The average age was 16 years (SD=4.1). Hence, an average TBM of 976 kg was used in the calculation of the food consumed by walruses in Young Sound.

Estimation of the walrus consumption of bivalves in Young Sound

For the calculation of bivalve biomass consumption by walruses inshore in Young Sound it was assumed that a group of 40 walruses have access to the mollusk banks west of Sandøen during an open-water season usually lasting about 90 days. Given the average fraction of the total time spent by the walruses "at sea" inside this study area (ca. 30%), an estimated total of 1080 "walrus feeding days" are spent inshore in Young Sound (Table 22).

Method I
Based on the estimates of the daily mean number of dives to 6 m and deeper and number of dives of between 5 and 7 min duration, the walruses make a total of 127 400 to 195 480 feeding dives, in the Young Sound study area during the open water season.

Using the estimates on number of bivalves, SF dry matter and wet weight, consumed per feeding dive (Table 22), the estimates (two methods of determining number of feeding dives, Table 20 and Table 21) of the total number of bivalves consumed inshore in Young Sound during the open water season ranged from ca. 7 to ca. $10 * 10^6$ (Table 22). The estimates of the corresponding amounts of bivalve DM and WW were ca. 18 to ca. 29 tons and ca. 74 to ca. 114 tons, respectively (Table 22).

Method II
The daily mean gross food consumption was ca. 59 kg/walrus/24 wet hr. (95% CI: 41-73 kg/walrus/24 h) and the corresponding estimate of the total amount of SF bivalve WW consumed by walruses during the open water season was ca. 63 tons (Table 22). Daily feeding rates in walruses of 6-7% of TBM (Born et al. 2003, Acquarone et al. *this thesis*) indicate that the estimates of 63 tons and 74 tons are the most plausible.

Estimation of the impact of the walruses on the bivalve community

The estimates of consumption by walruses correspond to the removal during the open-water season of between ca. 2.2 g/m^2 SF bivalve WW (*Method I*), or 1.9 g/m^2 SF bivalve WW (*Method II*).

The standing biomass and annual production in Young Sound of the two important walrus food items *Mya* sp. and *Hiatella* sp. are substantial (biomass and production of *Serripes* sp. were not determined). Between 0 and 10 m depth, where tidal effects and ice scouring negatively influence on the density of the infauna, the SF WW biomass and annual production of these two species taken together are 27.9 g/m^2 and 1.26 g/m^2 * year, respectively. In the preferred foraging depths of walruses (i.e. >10 to ca. 30 m; Wiig et al. 1992, Gjertz et al. 2001, Born et al. 2003), the corresponding values are 266 to 281 g/m^2, and 13.3 to 17.4 g/m^2 * year, respectively (Sejr 2004).

Between >30 and 40 m, the biomass and production of *Mya* sp. and *Hiatella* sp. were ca. 155 g/m^2, and ca. 7.0 g/m^2 * year, respectively (Sejr 2004).

Table 20 Number of dives and percentage of dives made to different depth intervals by an adult male walrus (same individual in both seasons) inshore in the Young Sound area (NE Greenland) in 1999 and 2001.

				% of all dives				
ID	Dates	Month	Year	0-6 m	>6 m	No. dives	No. 6-hr blocks with dive data $^{1)}$	No. of dives beyond 6 m per 24 h$^{1)}$
6481	23-31	Aug.	1999	20.1	79.9	1798	29	198
	1-30	Sep.		17.7	82.3	4796	86	184
4344	24-31	Jul.	2001	24.6	75.4	1183	22	162
	1-2	Aug.		40.9	59.1	127	3	100
All	-		99+01	19.7	80.3	7904	140	181

1: periods of haul-out were excluded

Table 21 Number of dives and percentage of dives made in different intervals of dive duration by an adult male walrus (same individual in both seasons) inshore in the Young Sound area (NE Greenland) in 1999 and 2001.

			% of dives					
ID	Period	Year	5-7 min	4-8 min	Total no. of dives$^{1)}$	No. of 6-hr blocks monitored	No. of 5-7 min long dives per 24 wet h	No. of days monitored
6481	27 Aug. - 2.Oct.	1999	58.3	76.1	1335	27	142	10
4344	25 Jul. - 2 Aug.	2001	66.0	78.2	565	12	124	4
All		99+01	60.6	76.7	1900	39	118	14

1: Periods with haul-out excluded

Table 22 Estimates of bivalves, dry matter (DM) and shell-free (SF), wet weight (WW) consumed per dive, total number of walruses using Young Sound, and estimates of total amount of bivalves, DM and WW eaten in the study area by walruses during the open water season. During the open water period a total of 40 walruses used about 30% of their inshore for a total of 1080 "walrus feeding days".

	Parameter	Mean/estimated value	95% CI	Comments
A	No. Bivalves ingested/dive	53.2	43.0-64.4	Estimated from 10 feeding dives in Young Sound (Born et al. 2003)
B	DM/dive (g)	149.0	112.0-186.0	(Born et al. 2003)
C	WM/dive (g)	583.0	444.0-722.0	(Born et al. 2003)
D	Total no. walrus	40	-	Genetically identified (cf. Materials and methods)
E	No. walrus feeding days	1080	-	(duration of open water season, 90 d . % occupancy . 40 walrus)
F	No. dives/day	118-181	-	Based on Table 20 and Table 21
G	Total no. of bivalves eaten	$6.8 . 10^6$-$10.4 . 10^6$	-	(A . E . F)
H	Total DM eaten (.10^3 kg)	18-29	-	(B . E . F)
I	Total WW eaten (.10^3 kg)	74-114	-	(C . E . F)
J	Total WW eaten (.10^3 kg)	63	44-79	Based on mean TBM and a daily food consumption of 6% (95%CI: 4.2-7.5%) of TBM when in water (Born et al. 2003).

DM = Dry Matter; WW = Wet Weight, or wet matter

Figure 17 Movement of an adult male walrus (6481) in Young Sound and adjacent areas in NE Greenland between 23 August and 21 November 1999. Black signatures = locations received during the open water period until 3 October when a dense layer of fast ice had formed west of Sandøen. Gray signatures = locations received after formation of fast ice.

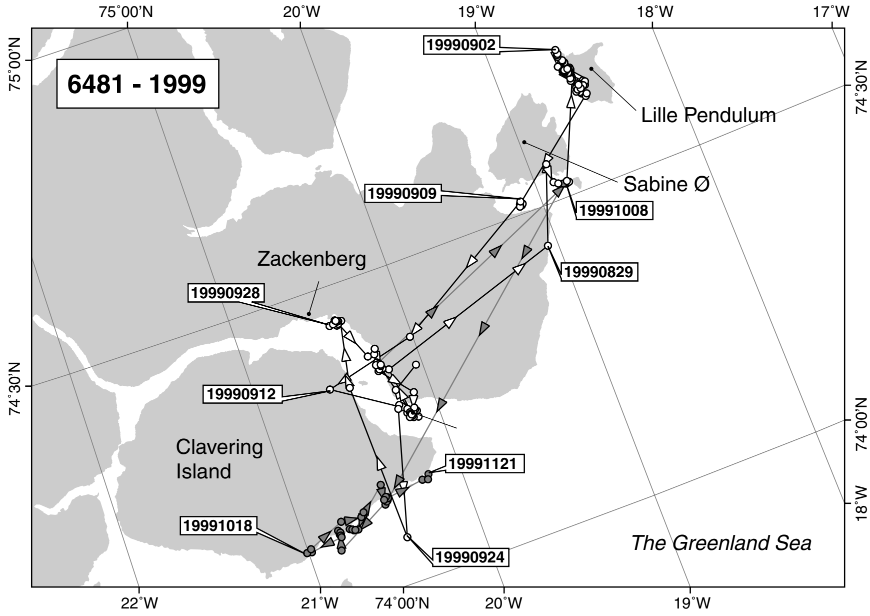


Figure 18 Movement of an adult male walrus (4344) in the Young Sound area (NE Greenland) between 24 July and 2 August 2001. This animal was tracked as ID no. 6481 in 1999 (Table 16, Figure 17). Locations were received until 3 September but only locations until 2 August remained after filtering (see Materials and methods).

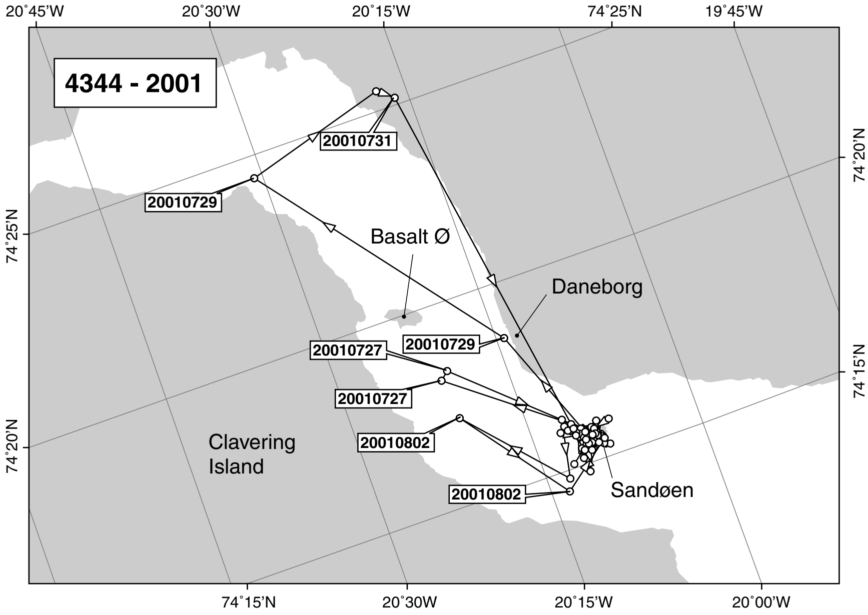
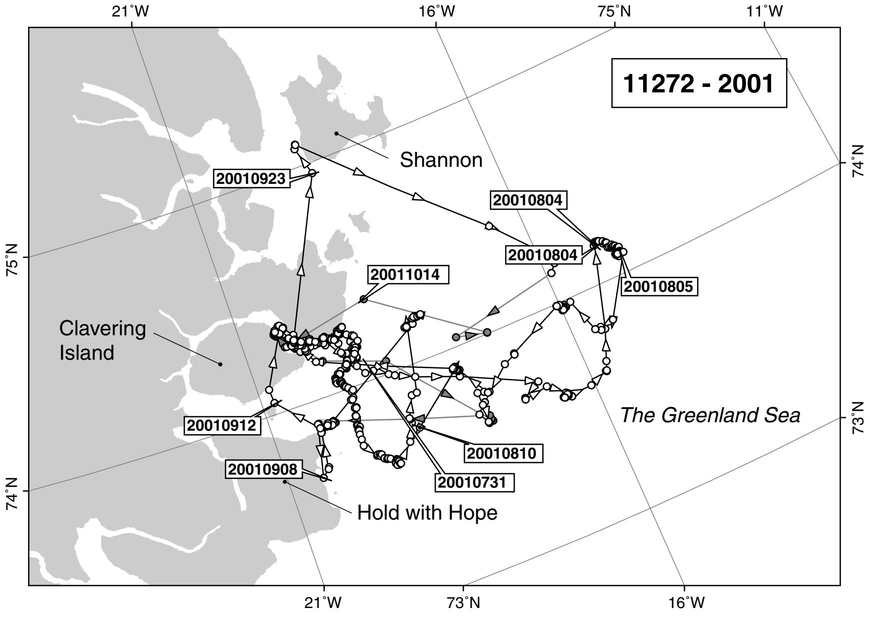


Figure 19 Movement of an adult male walrus (11272) in Young Sound and adjacent areas in NE Greenland between 27 July and 14 October 2001. Black signatures = locations received during the open water period until 3 October when a dense layer of fast ice had formed west of Sandøen. Gray signatures = locations received after formation of fast ice.



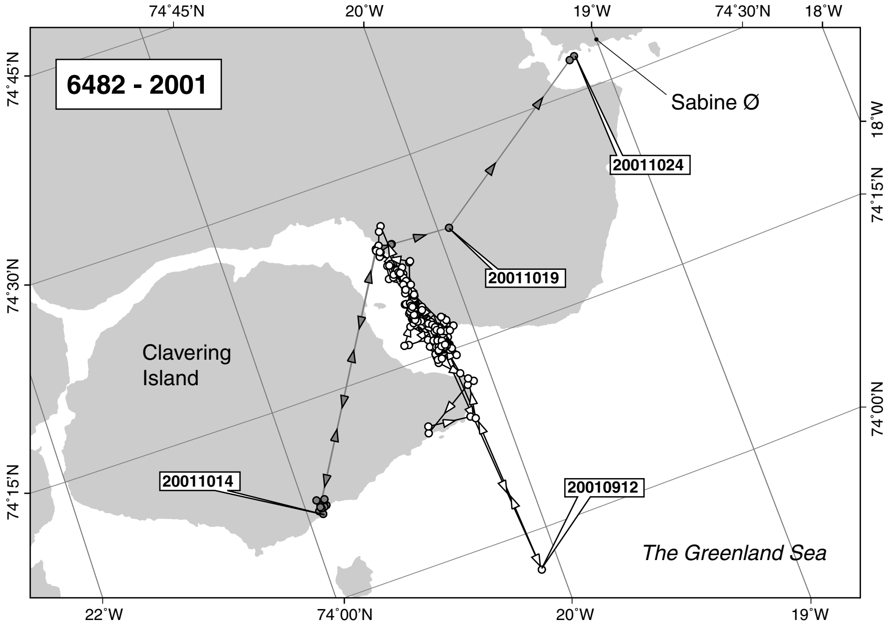


Figure 20 Movement of an adult male walrus (6482) in Young Sound and adjacent areas in NE Greenland between 28 July and 24 October 2001. Black signatures = locations received during the open water period until 3 October when a dense layer of fast ice had formed west of Sandøen. Gray signatures = locations received after formation of fast ice.

If adjusting for differences in bivalve densities at various depths (above) and size of the areas at different depths in Young Sound (Rysgaard et al. 2003: table 1), the standing biomass and annual production of *Mya* sp. and *Hiatella* sp. between 0 and 40 m average 165-173 g/m^2, and 8.0 to 9.8 g/m^2, respectively.

Our estimates of walrus predation during the open water season amount to 1.2-1.3% of the standing biomass of *Mya* sp. and *Hiatella* sp., and 19-28% of the annual production of these bivalves.

Discussion

Movement

The area of interest to the multi-disciplinary study of marine productivity has been defined as particularly the areas between Sandøen and Basalt Ø (Rysgaard & Glud 2004). This is also the area in which a variety of marine biological studies have been conducted since 1995 (e.g. Rysgaard et al. 1996, 1998, 1999, Sejr et al. 2000, 2002) and for which an overall "carbon/energy" flow-budget will be calculated (Rysgaard & Glud 2004). However, the general scarcity of "at sea" locations received from the walruses tracked in the present study only allowed for the determination of time in Young Sound west of Sandøen to Zackenberg and not a sub-division of the time-budget in this area. The satellite-derived locations during the four "walrus seasons" indicated that walruses on average spend about 30% of the open water season inshore in Young Sound with a clear preference for the northern shore west to Zackenberg.

During the study period the animals also used other feeding locations south, east and north of Young Sound. The latter area (i.e. Sabine Ø – Lille Pendulum Ø area) is a well-known walrus feeding habitat (Born et al. 1997, and references therein). In other studies of movement during summer, the walruses also regularly moved ca. 80 km or farther away from the haulout (Born & Knutsen 1992, Hills 1992, Wiig et al. 1996). Clearly, walruses use several alternative feeding grounds in the vicinity of their traditional haulout.

Usually the fraction of good quality locations received from walruses that are hauled out is relatively high whereas fewer locations usually of lower precision are received from animals that are at sea (Born & Knutsen 1992, Jay & Garner 2001). Walruses spend proportionally much time submerged (e.g. Wiig et al. 1992, Born & Knutsen 1997, Born et al. 2003) and do not always get the salt-water switch of the transmitter out of the water when ventilating (Born, unpubl. data), resulting in no or to few signals being transmitted. This may imply that relatively few locations are transmitted from areas where walruses are at sea and actively foraging. This fact obviously will influence the ability to proportionate time in different areas based on locations. On the other hand, when subtracting cases when the animals were hauling out, we believe that a general picture of relative time spent in various areas "at sea" could be deduced from the locations.

Haul-out and diving activity

We tracked relatively few animals during two open water seasons. However, the activity of the animals that were monitored via satellite telemetry was very typical of walruses in general. The animals hauled out for an average of about 31% of the time, which is in close agreement with haul-out times obtained during August-September in other studies of walrus activity involving satellite telemetry (Hills 1992, Born & Knutsen 1992).

We defined dives >6 m as feeding dives. The proportions of the number of dives to these depths were within the range observed in walruses that were studied in August in Dove Bay in 2001. Six adult male walruses equipped with MK-7 dive-recorders (Wildlife Computers) hauled out for an average of 34% of the time and made an average of 165 dives >6 m/24 "wet" hr. (range: 108-208 dives/24 h) (Acquarone et al. *this thesis*).

In Young Sound bivalve food items are mainly distributed between 10 and 40 m depth and are less abundant at depths less than ca. 6 due to ice scouring (Sejr 2002, 2004). Visual observations of walruses feeding along the northern coast of Young Sound indicate that they feed between ca. 8 and ca. 34 m (Born et al. 2003). The vast majority of time at depth and dives were between 6 and 42 m which is typical of walruses that are though to be feeding (Gjertz et al. 2001, Jay et al. 2001). Hence, our assumption that activity between 0 and 6 m was mainly associated with traveling, breathing and resting at the surface, and social interactions with other walruses seems sound.

We also quantified the foraging from the number of dives of 5-7 min duration, which is the duration of typical feeding dives in walruses (Wiig et al. 1992, Gjertz et al. 2001, Jay et al. 2001).

In the present study the walruses made an average of 118-181 presumed feeding dives/24 hr. in the water. Adding "at surface" intervals of ca. 1 min between feeding dives (Born & Knutsen 1997, Born et al. 2003) indicate that the walruses were engaged in diving for food for between 58% (dive duration) and 88% (dives at depth) of their "in water" time. Six adult male walruses that were tracked by use of satellite transmitters in Dove Bay in 1989 were diving for an average of 72% of their "at sea" time (range: 65%-77%; Born & Knutsen 1990b). Similarly, six males studied with MK-7 dive-recorder in the same area were submerged for an average of 66% of the time (range: 34%-84%; Acquarone et al. *this thesis*). This indicates that (1) the activity seen in the present study is typical of walruses when feeding inshore, and (2) that the estimate of total food consumption based on number of 5-7 min dives/24 wet hr. is plausible, and likely more so than the estimate based on number of dives deeper than 6 m/24 wet hr.

It can be assumed that not all dives deeper than 6 m and 5-7 min long resulted in successful feeding. In that case, the amount of food consumed by the walruses is here overestimated to an unknown extent.

Estimates of number of walruses, TBM and individual food consumption

In the calculations of the number of "walrus feeding days" we assumed that the total group using the area is about 40 adult males. This was based on the genetic identification of individuals in 2002. However, daily maximum counts of nearly 50 walruses have been made at Sandøen in some years. Therefore, using 40 and not 50 for the size of the group leads to a 20% under-estimation of the predation pressure exerted by walruses on the benthic fauna in Young Sound.

Based on the tracking of admittedly few animals we found that walruses on average spend about 30% of their time in the study area, resulting in an estimate of about 12 animals foraging in Young Sound at any given time. However, opportunistic observations made by researchers and photographers during late July – August 2001 and 2002 indicate that usually fewer walruses are found in Young Sound west of Sandøen. Hence, we can not preclude that our estimate of walrus consumption in the study area is over-estimated by an unknown factor.

We estimated that the average TBM of the walruses in Young Sound is about 976 kg. This is somewhat higher than average TBM of 512 kg used by Welch et al. (1992) for calculation of walrus feeding in Lancaster Sound (Canada), and 712 kg used by Fay (1982) in Alaska. However, the walruses that feed in Young Sound are nearly all adult males in contrast to the other two areas where all age classes and both sexes are represented at the summer feeding grounds.

There are indications that the growth equation used in the present study tend to under-estimate the TBM of walruses >ca. 18 years of age (Figure 15). If using the alternative growth model in Figure 15, the estimate of average TBM of walruses in Young Sound became 922 kg. Consequently, the estimate of walrus consumption using *Method II* would be about 7% lower.

For calculation of food consumption (*Method II*) we assumed that the daily gross food intake of walruses in the water is 6% (Born et al. 2003). Fay (1982:table 25) assumed that the daily food consumption of a 1000 kg walrus is 5.7%. Direct measurement of walrus energy expenditure in NE Greenland in 2001, indicated that daily gross food consumption in adult male walruses is at least 7% of TBM (Acquarone et al. *this thesis*). We therefore believe that the estimate of 6% used in this study is realistic.

The impact of the walruses on the bivalve community

A high standing stock of bivalves is present in the study area (Sejr 2004, Sejr et al. 2000, Sejr et al. 2002), which is representative of other inshore ice-covered Arctic areas (Berthelsen 1937, Vibe 1950, Ockelmann 1958, Thomson et al. 1986, Grebmeier et al. 1989, Welch et al. 1992). The estimates of walrus consumption in Young Sound were 1.3% (highest rate) of the standing biomass of the two important food items *Mya* sp. and *Hiatella* sp., and 28% of their production. These estimates were based on the highest of daily dive rate. However, the more plausible average dive rate of 118/24 wet h, and the method using daily gross feeding rate and TBM, indicated that the consumption was considerably lower.

At present there is apparently enough food for walruses in Young Sound. However, if all the walruses at Sandøen (50) changed their foraging strategy and used every day of the entire open water season (90 d) foraging in Young Sound their consumption would only amount to 8-9 g SF WW/m^2, corresponding to ca. 5% of the standing biomass of the two bivalves, and all of their annual production.

However, the standing stocks and productivity in Young Sound of other walrus food items, for example *S. groenlandicus*, have not been determined. If these are also considered, the inshore bivalve banks in Young Sound represent a richer food source than accounted for in our calculation of predation, which is based only on two prey species.

The estimates of gross food intake per dive or per TBM used in the present study were adopted from Born et al. (2003). The estimates were based on three bivalve species that constitute the far most important portion of the walrus diet. However, walrus feed on a variety of benthic food (e.g. Fay 1982) and as pointed out by Born et al. (2003) it cannot be precluded that walruses in Young Sound also feed on other bivalves (e.g. *Astarte* sp.) and invertebrate benthos (e.g. polychaetes, sea cucumbers and gastropods) other than the three species considered. On the other hand, historical observations of the diet of walruses feeding in the vicinity of Young Sound indicate that *M. truncata* and *Hiatella* sp. were principal food items (Peters 1874, Payer 1877a,b) and are also the most abundant species in the area. Hence the estimates of the present study of walrus ingestion rates inferred from the bivalves studied are likely to be representative.

We conclude that (1) walruses that haul-out on Sandøen only use Young Sound as one of several alternative feeding grounds during summer, and (2) that activity data and information on number of walruses and food ingestion rates indicates that walrus predation in

Young Sound is below carrying capacity of the Young Sound study area. This latter conclusion is supported by the fact that historically walruses were more abundant in the area (Born et al. 1997).

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The Field Metabolic Rate (FMR) of two free-living, adult, male Atlantic walruses (*Odobenus rosmarus*) was measured by the Doubly Labelled Water isotopic technique in NE Greenland. Estimates of FMR were 345 MJ day^{-1} for a 1,370 kg and 417 MJ day^{-1} for a 1,250 kg walrus (mean = 381 MJ day^{-1}) reflecting a difference in diving activity measured by ARGOS© Satellite-Linked Transmitters and Time-Depth Recorders. Converted to walrus food equivalents these quantities correspond to 68 and 82 kg day^{-1} mussel shell-free fresh matter, respectively, representing 5.0 and 6.6 % day^{-1} of their total body mass (TBM). These values agree with the estimates of bivalve mollusc intake derived from underwater, visual observations and activity data obtained by ARGOS© Satellite-Linked Dive Recorder of one walrus feeding in the wild (57 kg day^{-1} shell-free fresh matter, 4.7 % day^{-1} for TBM of 1226 kg). From this information and from data on local prey abundance, walrus predation in the Young Sound study area in NE Greenland appears to be lower than the carrying capacity. Deuterium Oxide dilution was employed on seven animals to estimate Total Body Water (TBW) content (56.8 % of TBM) and Water Turnover (44.5 g kg^{-1}day^{-1}) to obtain essential data for the calculation of the minimum effective dose of Doubly Labelled Water. TBW measures thus obtained and general allometric equations of body composition estimated body fat to 24.7 % of TBM which did not differ significantly from the estimates of blubber content obtained from the dissection of walruses in the wild. Chemical immobilization by etorphine HCl reversed by diprenorphine HCl has been employed to restrain the animals for instrument attachment. During isotope enrichment, the immobilization time has been prolonged to up to 6.75 hr. by use of medetomidine HCl.

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