

NORTH PACIFIC RESEARCH BOARD PROJECT FINAL REPORT

Ice Seal Movements and Stock Structure in a Changing Cryosphere

NPRB Project 515 Final Report

Brendan P. Kelly^{1,2}, Peter Boveng³, and Bradley R. Swanson⁴



¹ School of Arts and Sciences
University of Alaska Southeast
11120 Glacier Highway
Juneau, Alaska 99801

² Current address:
Office of Polar Programs
National Science Foundation
4201 Wilson Blvd.
Arlington, Virginia 22230

³ National Marine Mammal Laboratory
National Marine Fisheries Service, NOAA
7600 Sand Point Way
Seattle, Washington 98115-6349

⁴ Department of Biology
Central Michigan University
Mount Pleasant, MI 48859

Abstract

We monitored the movements of ringed seals using radio and ultra-sonic tags during the winter – spring period when the seals were occupying shorefast ice and using satellite-linked transmitters in summer and fall when the seals ranged away from their winter sites. In the shorefast ice habitat, the home ranges of 27 breeding males ranged from $< 1 \text{ km}^2$ to 13.9 km^2 ($\bar{X} = 1.50 \text{ km}^2$, $SD = 3.07$) while the home ranges of 28 breeding females ranged from $< 1 \text{ km}^2$ to 27.9 km^2 ($\bar{X} = 1.95 \text{ km}^2$, $SD = 5.17$). The 3-dimensional volumes used by 9 seals tracked acoustically under the ice ranged from 0.03 to 0.13 km^3 ($\bar{X} = 0.07$, $SD = 0.04$) for subadults and breeding males and from 0.10 to 0.17 km^3 ($\bar{X} = 0.13$, $SD = 0.04$) for breeding females. Three of the radio tracked seals and 9 tracked by satellite ranged up to 1800 km from their winter/spring home ranges in summer but returned to the same small sites during the ice-bound months in the following year. The restricted movements of ringed seals during the ice-bound season have implications for their foraging ecology, population structure, and conservation. We developed a new method of obtaining DNA samples from ice-associated seals. Skin cells shed on sea ice were a viable source of DNA for microsatellite analysis. This source of DNA should allow the rapid collection of a large number of noninvasively collected DNA samples in ice-breeding phocids.

Keywords: Ringed seal, *Phoca hispida*, Arctic Ocean, population genetics, home range

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Study Chronology

April through June 2005

In the first months of this project, we captured and attached satellite-linked and conventional VHF radio tags to the hind flippers of 11 ringed seals (*Phoca hispida*) and 2 bearded seals (*Erignathus barbatus*) near Barrow, Alaska. We also extracted DNA from skin samples from live seals and from around breathing holes on the ice.

July through December 2005

We continued to track seals via satellite-linked transmitters attached to 11 ringed seals and 2 bearded seals. We began preliminary analysis of time spent out of the water by the tagged seals. We analyzed DNA at 8 microsatellite loci for 54 ringed seals. We also presented on the project in seven local, national, and international venues.

January through June 2006

We continued to track by satellite seals tagged in 2005 and 12 additional seals tagged in 2006. In May 2006, we collected an additional 28 samples of shed ringed seal skin between Peard Bay and Point Barrow. We also identified 1,503 ringed seal specimens collected during the breeding season (March-June) and archived at the University of Alaska Museum of the North. The study progress was presented at 16 meetings between January and June 2006.

July through December 2006

During the second half of 2006, we continued to track ringed seals tagged with satellite-linked transmitters in 2005 and in 2006. We focused our genetic analyses on samples collected during the breeding season. In August 2006, we extracted cheek teeth from 162 museum specimens, and we recovered DNA from those samples. We began analysis of microsatellite DNA at Swanson's laboratory (Central Michigan University) and of mtDNA in Tallmon's laboratory (University of Alaska Southeast). Results were presented at 13 meetings.

January through June 2007

In May 2007, we attached satellite-linked transmitters to two ringed seals captured in their breeding sites in Kotzebue Sound. The 2007 tagging was done in collaboration with the Native Village of Kotzebue. In 2007, we added 264 additional DNA samples to the 151 samples collected in 2005 and 2006. The additional skin samples came from the ice in Kotzebue Sound and near Point Oliktok (Beaufort Sea) as well as archived specimens from three other sites. Results of this study were presented in 11 venues between January and July 2007.

July 2007 through April 2008

We continue to track seals tagged with satellite-linked transmitters and have expanded on our genetics study in NPRB Project 631.

Introduction

Prior to the recent declines, sea ice seasonally extended to $14 \times 10^6 - 16 \times 10^6 \text{ km}^2$ in the northern hemisphere (Overland and Wang 2007; Serreze et al. 2007; Comiso et al. 2008). That ice cover supports an ecosystem that includes seven species of pinnipeds that depend on ice as a breeding platform (Davies 1958; Burns 1970; Fay 1974). At least four other pinniped species rest on the ice opportunistically. The ice-associated seals are the main prey of polar bears and have been vital to the subsistence economy of northern people for thousands of years. The sea ice ecosystem and subsistence economies in the Arctic are threatened by observed and predicted decreases in sea ice cover associated with climate change. Assessing the impacts on the ice-associated seals requires knowledge of their movement patterns and population biology. A better understanding of the population biology and population structure of ice-associated seals will help the NMFS and Alaska Natives to manage and protect these important components of the sea ice ecosystem.

Fundamental to the vulnerability of seal populations to extinction is degree to which they are subdivided into demographically distinct subpopulations (Dizon et al. 1992). If immigration occurs from other populations, then the vulnerability is low, but if immigration is absent or very rare, then the potential for local extinction is high. In the course of another investigation, we observed 3 ringed seals returning in successive years to the same small breeding sites in the shorefast ice of the Beaufort Sea (Kelly 2006). Those observations were suggestive of natal philopatry and limited gene flow, but they were based on local observations that would be difficult to repeat in ice-covered seas. Therefore, in this study, we developed two novel methods to investigate ringed seal movements and population structure.

Historically, researchers have attached telemetry devices to the hair of seals using epoxy adhesives. That method has provided a great deal of useful information but limits the record to movement made between the time of deployment and the annual molt. We pioneered the use of small satellite-linked transmitters attached to the hindflippers of seals by way of plastic tags. Attaching the tags to the webbing of the hindflippers and programming them to maximize battery allowed us to collect movement data spanning two annual breeding seasons.

At the outset, we recognized that fidelity to breeding sites by adult seals was a necessary but insufficient condition to limit gene flow and generate local subpopulations. Additionally, we need to know whether those breeding seals are returning to their own birth sites to breed. Telemetric

tracking from birth to sexual maturity (4 – 7 years) is impractical, and we sought molecular evidence of natal philopatry. Again, standard approaches of taking biopsies from living or harvested seals were limited in the sea ice environment, and we sought to determine whether DNA could be reliably sampled from shed skin found on the ice.

Overall Objectives

(1) Use satellite-linked transmitters to test the null hypothesis that ringed seals are equally likely to breed at any location visited during the year, versus the alternative hypothesis that a breeding location in one year will be a good predictor of a subsequent breeding location.

(2) Collect shed ringed seal skin and hair from the ice in two or more locations and determine the most suitable protocols for obtaining DNA from such samples.

The inter annual movements of ringed seals recorded by satellite tracking are combined in chapter one with data on under-ice movements during the breeding season and records of on-ice resting sites in the breeding period to present a comprehensive view of ringed seal movements and potential gene flow. In the second chapter, we present the protocol developed in this study for collecting DNA from shed skin samples (Swanson et al. 2006). That method is being employed in another NPRB project (631) to further investigate the population structure of ringed seals in the Arctic Ocean.

Chapter 1- Seasonal home ranges and fidelity of breeding sites among ringed seals

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SEASONAL HOME RANGES AND
FIDELITY TO BREEDING SITES AMONG RINGED SEALS

Brendan P. Kelly^{1,2}, Oriana H. Badajos^{1,3}, Mervi Kunnasranta^{1,4}, John R. Moran^{1,5},
Micaela Ponce¹, Douglas Wartzok⁶, and Peter Boveng⁷

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¹ Department of Natural Sciences
University of Alaska Southeast
11120 Glacier Highway
Juneau, Alaska 99801

² Current address:
Office of Polar Programs
National Science Foundation
4201 Wilson Blvd.
Arlington, Virginia 22230

³ Current address:
Alaska Department of Fish and Game
Homer, Alaska

⁴ Current address:
Finnish Game and Fisheries Research Institute
Turku Game and Fisheries Research
Itäinen Pitkätatu 3
FIN-20520 Turku, Finland

⁵ Current address:
Alaska Fisheries Science Center, NOAA Fisheries
Ted Stevens Marine Research Institute
17109 Pt Lena Loop Rd
Juneau AK 99801

⁶ Florida International University
University Park PC 526
Miami, FL 33199

⁷ National Marine Mammal Laboratory
National Marine Fisheries Service, NOAA
7600 Sand Point Way
Seattle, Washington 98115-6349

ABSTRACT

We monitored the movements of ringed seals (*Phoca hispida*) using radio and ultra-sonic tags during the winter – spring period when the seals were occupying shorefast ice and using satellite-linked transmitters in summer and fall when the seals ranged away from their winter sites. In the shorefast ice habitat, the home ranges of 27 breeding males ranged from $< 1 \text{ km}^2$ to 13.9 km^2 ($\bar{X} = 1.50 \text{ km}^2$, $SD = 3.07$) while the home ranges of 28 breeding females ranged from $< 1 \text{ km}^2$ to 27.9 km^2 ($\bar{X} = 1.95 \text{ km}^2$, $SD = 5.17$). The 3-dimensional volumes used by 9 seals tracked acoustically under the ice ranged from 0.03 to 0.13 km^3 ($\bar{X} = 0.07$, $SD = 0.04$) for subadults and breeding males and from 0.10 to 0.17 km^3 ($\bar{X} = 0.13$, $SD = 0.04$) for breeding females. Three of the radio tracked seals and 9 tracked by satellite ranged up to 1800 km from their winter/spring home ranges in summer but returned the same small sites during the ice-bound months in the following year. The restricted movements of ringed seals during the ice-bound season have implications for their foraging ecology, population structure, and conservation.

Key words: breeding, home range, population structure, site fidelity, telemetry

INTRODUCTION

The seasonal movements and population structure of ringed seals (*Phoca hispida*) have not been documented previously, in part, because of the species' wide range encompassing a difficult environment, the seasonally ice-covered seas (and some freshwater lakes) of the Northern Hemisphere (McLaren 1958; Reeves 1998). Inuit and other northern people have long known that ringed seals occupy seasonal sea ice from the time of ice formation in the fall through break-up the following summer (Boas 1888; Taylor 1968; Cox and Spiess 1980; Sabo and Jacobs 1980). The ringed seal's use of breathing holes abraded in the ice and subnivean lairs (snow caves) excavated in the snow permit them to remain in areas of otherwise solid ice cover throughout the winter and the spring, when they give birth and suckle their young under the snow (Hall 1866; Chapskii 1940; McLaren 1958). Inuit hunters and field biologists inferred movements of seals based on the distribution of breathing holes and lairs (McLaren 1958; Smith and Stirling 1975). Direct evidence of movements during the spring months was first provided by radio tracking studies (Kelly and Quakenbush 1990). Fragmentary information suggested that Arctic ringed seals ranged over wide areas in summer (Smith 1987; Heide-Jørgensen *et al.* 1992; Kelly 1996; Kapel *et al.* 1998; Ridoux *et al.* 1998; Teilmann *et al.* 1999) and that interbreeding took place over large areas (Scheffer 1958; King 1983; Davis 2004). Ringed seals inhabiting the Baltic Sea and Lake Saimaa in Finland, however, apparently do not make long distance movements and

exhibit strong fidelity to particular sites (Hyvärinen *et al.* 1995, Härkönen *et al.* 1998; Kunnasranta 2001, Koskela *et al.* 2002). Here, we report on the extent of seasonal movements by Arctic ringed seals.

Better knowledge of the ringed seal's seasonal movements and patterns of habitat use are important to understanding their relationships to (1) polar bears (*Ursus maritimus*) and other predators, (2) expanding industrial development, and (3) rapid environmental change, especially the decreasing duration of season snow and ice cover (Kelly 1988, 2001; Tynan and DeMaster 1997; Stirling and Smith 2004; Ferguson *et al.* 2005).

Ringed seals have frequently been described (e.g., McLaren 1958; Burns 1970) as showing a preference for shorefast sea ice or as occurring in higher densities there than in the moving pack ice. Many ringed seals, however, do breed in pack ice, and we are unable to find strong support for assertions that they preferentially occupy shorefast ice. Nonetheless, for logistical reasons, our study focused on seals breeding in the latter habitat.

Ringed seals rest, give birth, nurse their young, and molt on seasonal sea ice. In winter and spring, they feed under the ice, and in summer and early fall, they feed either in open water or under ice. Thus, we divided our investigation of movements and habitat use into three time periods each corresponding to an important aspect of the seals' life history. We refer to the *subnivean period* as the period in early winter through mid May to early June when seals in our study areas rested on the ice primarily in subnivean lairs. Lairs likely are excavated as soon as sufficient snow has accumulated on the ice and have been documented as early as January (Smith *et al.* 1994). Our investigation of activity in the subnivean period focused on April to mid June, a time that is especially important to reproductive success as it is then that the pups are born and nursed.

Between abandonment of the lairs in May or June and ice break-up (typically in July in our study areas), ringed seals rested without cover on the ice. At that time, direct exposure to solar radiation is important to the elevated skin temperatures necessary for epidermal regeneration (Feltz and Fay 1966). We refer to the period between lair abandonment and ice break-up as the *basking period*.

From ice break-up to the subsequent freeze-up in fall, ringed seals forage more intensively (Lowry *et al.* 1980; Weslawski *et al.* 1994) and are less frequently observed out of the water. We

refer to that period as the *foraging period* with the recognition that ringed seals also forage during the breeding season (Kelly and Wartzok 1996) in contrast to most species of phocid seals (Costa 1991).

Our study of ringed seal movements and habitat use relied on three types of data:

- 1) on-ice resting locations in the subnivean and basking periods – primarily April, May, and June - determined by tracking seals carrying VHF radio transmitters,
- 2) under-ice movements in those periods determined by tracking seals acoustically, and
- 3) on-ice resting locations throughout the year determined by tracking seals carrying satellite-linked transmitters.

METHODS

Ringed seals were captured in breathing holes in April or May during 11 field efforts between 1990 and 2003. The breathing holes and subnivean lairs were located by dogs trained to alert to the odour of ringed seals (Smith and Stirling 1975; Kelly and Quakenbush 1987). Seals were live-captured in breathing holes using a remotely triggered version of the net described by Kelly (1996).

We determined the on-ice resting location of 60 ringed seals captured near Prudhoe Bay, Alaska (70° 29.1'N, 148° 21.4'W) in April, May, and early June 1999, 2000, 2001, and 2003 by radio tracking. We glued a 26 g VHF radio transmitter (Advanced Telemetry Systems, model MM170) with a unique frequency to the hair on each seal's back. To extend our observations of seal haulout bouts (time spent out of the water) and movements beyond the annual molt, we attached a second 23 g VHF radio transmitter (Advanced Telemetry Systems, model MM420) to one hind flipper of each seal by way of a cattle ear tag (Temple Tag, Inc., model 73200) in 2002 and 2003.

We monitored radio signals hourly from stations equipped with 8-element Yagi antennas on 11 m high masts and within 5 km of the seal capture sites. We rotated the antenna through 360° while monitoring and recorded the direction from which each signal was received. Each time a seal came out of the water, as indicated by the presence of its radio signal, we determined its location using a mobile receiver and hand-held directional antenna array. The directional antenna array consisted of two Yagi antennas communicating with the acoustic receiver by way of a null

combiner. Thus, the bearing from the array to a transmitter was indicated by a null surrounded by high amplitude signals. Typically, five or more bearings (with an accuracy of approximately $\pm 3^\circ$) from points surrounding a tagged seal were obtained, and the seal's position was read as the intersection of those bearings. Once the seal's position was determined, we recorded whether each seal was concealed within a lair or visible on the ice surface.

The locations of subnivean lairs and basking sites (sites where seals rested on the ice without snow cover) used by the tagged seals were plotted using MapSource (Garmin Corporation), transferred to an ArcView shape file, and the home ranges were delineated as minimum convex polygons (Worton 1987). The areas of those polygons were calculated to estimate home range sizes. The estimates did not account for water depth which did not exceed 9 m in any the areas used by seals in this portion of the study.

Diel patterns of haulout bouts were examined over periods ranging from 22 to 52 days for each tagged seal. The frequency distributions of haulout bouts at each hour of the day were plotted and the circular mean and standard deviations were calculated (Batschelet 1981). The frequency and duration of haulout bouts were examined separately for haulout bouts in lairs and haulout bouts at basking sites. The probability that the timing of haulout was the same when seals were in lairs and when they were basking was tested using Watson's U^2 statistic (Batschelet 1981). Rao's Spacing Statistic (U) was used to evaluate deviations from uniformity in the times at which seals were out of the water (Batschelet 1981).

Thirteen seals were tracked under the ice in Resolute Bay, Canada ($74^\circ 35'N$, $95^\circ 7'W$) in 1990, 1991, 1992, 1996, and 1997. Ultra sonic transmitters glued to each seal broadcast at unique frequencies (50 – 75 kHz) received via an array of four hydrophones suspended under the ice (Wartzok et al. 1992; Kelly and Wartzok 1996; Simpkins et al. 2001). A time difference of arrival algorithm displayed and recorded the three-dimensional position of each seal at 1 – 2 sec intervals (Wartzok et al. 1992). Nine of the seals tracked under the ice (2 breeding males, 3 breeding females, and 5 immature seals) were tracked continuously for 7 to 30 days. The three-dimensional, under-ice home ranges of those seals were calculated as convex hulls, the smallest convex set that contains all of the locations occupied (Barber et al. 1996).

We measured overlap in under-ice ranges for four pairs of seals that were tracked within the same area and time. We sampled the three-dimensional locations of those seals at 10 minute intervals (sufficient time for them to swim to any point in their under-ice home range) and then grouped

the observations in-to 200 x 200 x 50 m blocks. The size and shape of the blocks were chosen to ensure that some cells contained locations while not obscuring the shape of the home range (Doncaster 1990). For each pair of seals tracked within a single hydrophone array, we recorded the percentage of blocks used by either or both seals that was used by both seals.

Satellite-linked transmitters (Wildlife Computers, SPOT4 or SPOT-5 tags) were attached to the hindflippers of 25 ringed seals and 2 bearded seals captured in the breeding season in 2005, 2006, and 2007 (Table 1). The tags, programmed to transmit intermittently for one year or more, were attached to the webbing of the flipper by way of cattle ear tags (Temple tag). Conductivity switches turned the transmitters off while in the water. The tags transmitted location information and activity cycles (hourly percent time in versus out of the water) to ARGOS satellites. Tags were placed on 6 adult male, 1 subadult male, and 9 adult female ringed seals and a yearling male and a juvenile female bearded seal along the Chukchi Sea coast of Alaska (70.82°N 158.3°W) in April and May 2005 and 2006; on 4 adult male and 3 adult female ringed seals in the Canadian Beaufort Sea (69.64°N 136.5°W) in March 2006; and on 1 adult and 1 subadult male ringed seals in Kotzebue Sound (66.76°N 162.54°W) in May 2007.

RESULTS

Subnivean period

The location of each of 60 ringed seals was determined 1 to 32 ($\bar{X} = 12.8$, $SD = 7.02$) times by tracking their radio signals when they rested on the ice during the subnivean and basking periods. The number of resting sites identified for each seal ranged from 1 to 8 ($\bar{X} = 3.4$, $SD = 1.53$) and likely underestimated the actual number of holes used, as the seals' locations were not determined for every resting bout. The number of holes used increased with the number of times sites were identified and reached an asymptote at 4.5 holes per seal (Figure 1).

Home range size was estimated for 55 of the seals tracked during the subnivean period in 1999–2003 (Figures 2–6). The home ranges of 27 breeding males ranged from $< 1 \text{ km}^2$ to 13.9 km^2 ($\bar{X} = 1.50 \text{ km}^2$, $SD = 3.07$) while the home ranges of 28 breeding females ranged from $< 1 \text{ km}^2$ to 27.9 km^2 ($\bar{X} = 1.95 \text{ km}^2$, $SD = 5.17$). Most (94%) of the home ranges were less than 3 km^2 (Figure 7). Two of the larger home ranges included lairs $\geq 7 \text{ km}$ apart between which seals moved

repeatedly. In 2000, the home range of an adult female seal (OC00) included two lairs 8.6 km apart, and in 2001, an adult male (MK01) used two lairs separated by 7 km. Nine seals were

Table 1. Dates of first and last locations received from 25 ringed seals tracked by satellite in 2005 – 2007. Seals were captured in the Chukchi Sea (Peard Bay and Kotzebue Sound) and in the Beaufort Sea (north of Inuvik). Ad. = adult; Juv. = juvenile.

Seal	Capture site	Age / sex	First location	Last location
VK	Peard Bay	Ad. ♀	01 May 05	21 Jul 05
LY	Peard Bay	Ad. ♀	03 May 05	22 Jan 06
AS	Peard Bay	Ad. ♀	25 May 05	15 Mar 06
IO	Peard Bay	Ad. ♀	21 May 05	13 Jul 05
SI	Peard Bay	Ad. ♀	29 May 05	25 Aug 05
SJ	Peard Bay	Ad. ♀	25 May 05	09 Oct 05
TT	Peard Bay	Ad. ♂	21 May 05	27 Feb 06
IB	Peard Bay	Ad. ♂	17 May 05	09 Jul 06
SS	Peard Bay	Ad. ♀	25 May 05	05 Aug 05
SE	Peard Bay	Juv. ♂	25 May 05	17 Jun 05
JJ	Peard Bay	Ad. ♂	25 May 05	21 Jun 06
P1	Inuvik	Ad. ♂	20 Mar 06	21 Jun 06
P2	Inuvik	Ad. ♂	21 Mar 06	23 Mar 06
P3	Inuvik	Ad. ♂	13 Apr 06	29 Apr 06
P4	Inuvik	Ad. ♂	21 Mar 06	25 Jun 06
P5	Inuvik	Ad. ♀	01 Apr 06	25 Jun 06
P6	Inuvik	Ad. ♀	05 Jun 06	09 Jun 06
P7	Inuvik	Ad. ♀	26 Mar 06	17 Jun 06

Seal	Capture site	Age / sex	First location	Last location
AM	Peard Bay	Ad. ♂	28 Apr 06	02 Nov 06
BM	Peard Bay	Ad. ♂	13 May 06	07 Jun 07
AF	Peard Bay	Ad. ♀	07 May 06	26 Mar 07
CM	Peard Bay	Ad. ♂	19 May 06	17 Jan 07
BF	Peard Bay	Ad. ♀	19 May 06	05 Jun 07
JM	Kotzebue Snd.	Ad. ♂	19 May 07	13 Jun 07
JS	Kotzebue Snd.	Juv. ♂	25 May 07	02 Dec 07

Figure 1. Mean (and SE) of the number of holes used by radio tagged ringed seals as a function of the number of times haulout locations were determined. The asymptote is 4.5 holes per seal.

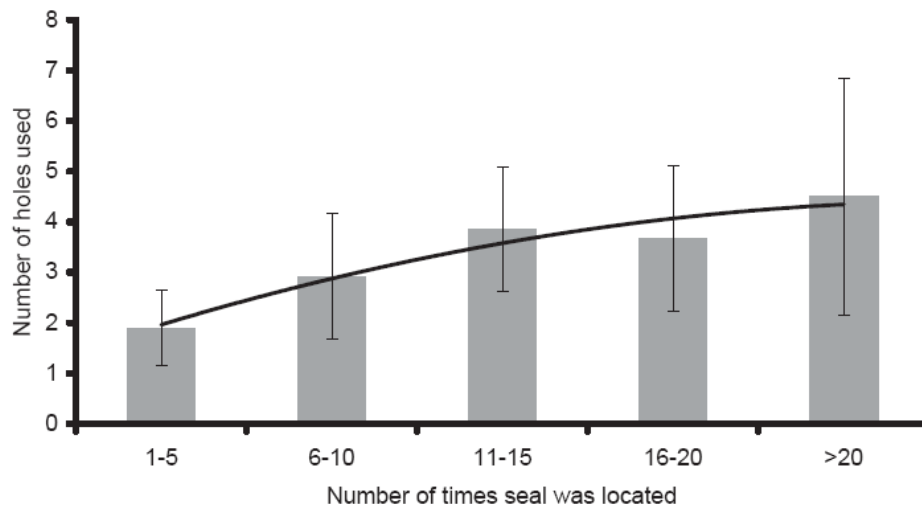


Figure 2. Locations of subnivean breathing holes and lairs located by trained dogs in 1999. The minimum convex polygons delineate the home ranges of 8 ringed seals based on on-ice resting sites. Also shown is the monitoring camp.

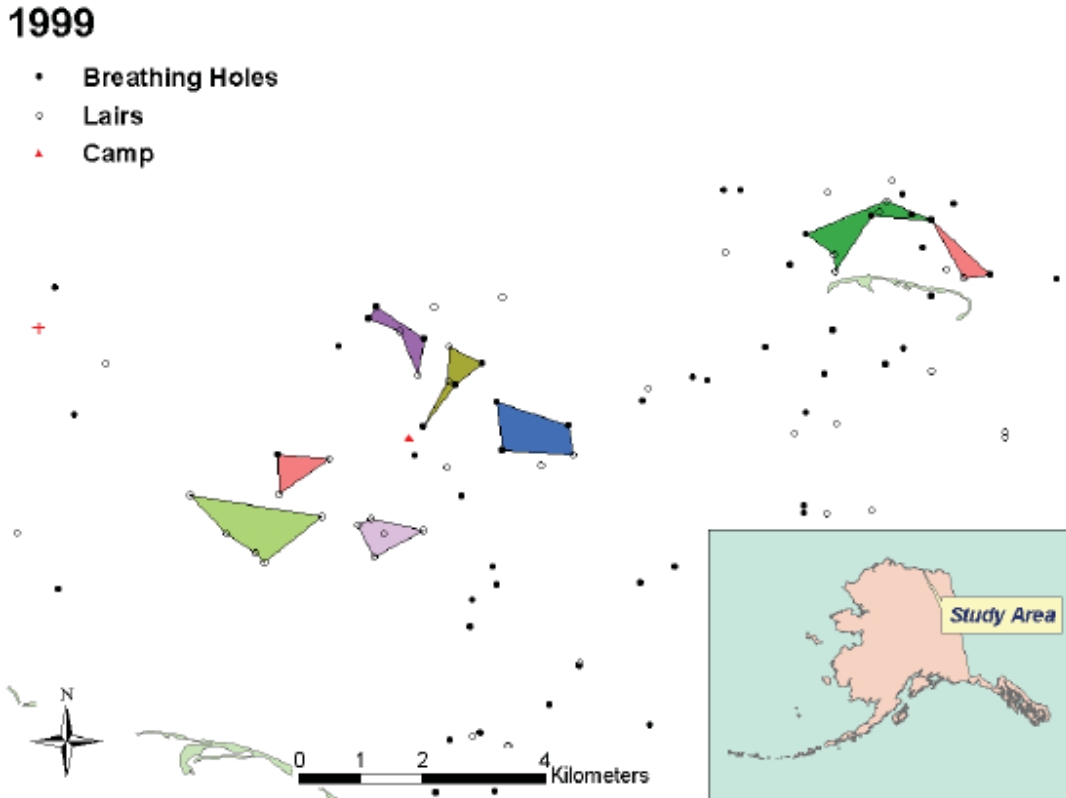


Figure 3. Locations of subnivean breathing holes and lairs located by trained dogs in 2000. The minimum convex polygons delineate the home ranges of 8 ringed seals based on on-ice resting sites. Also shown is the monitoring camp.

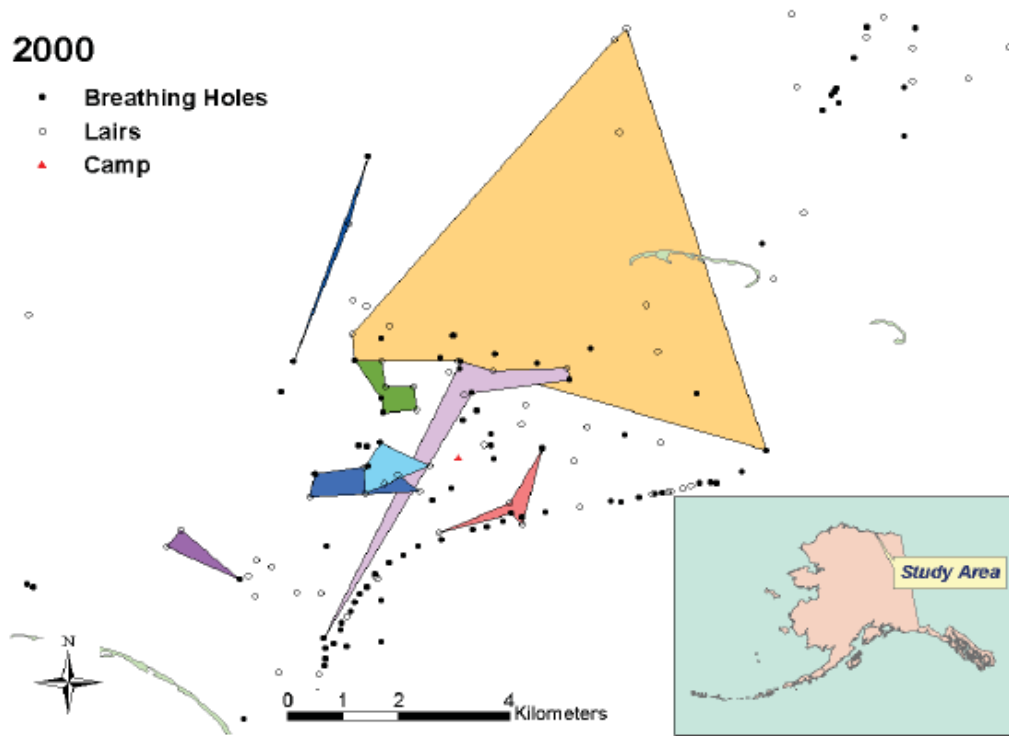


Figure 4. Locations of subnivean breathing holes and lairs located by trained dogs in 2001. The minimum convex polygons delineate the home ranges of 14 ringed seals based on on-ice resting sites. Also shown is the monitoring camp.

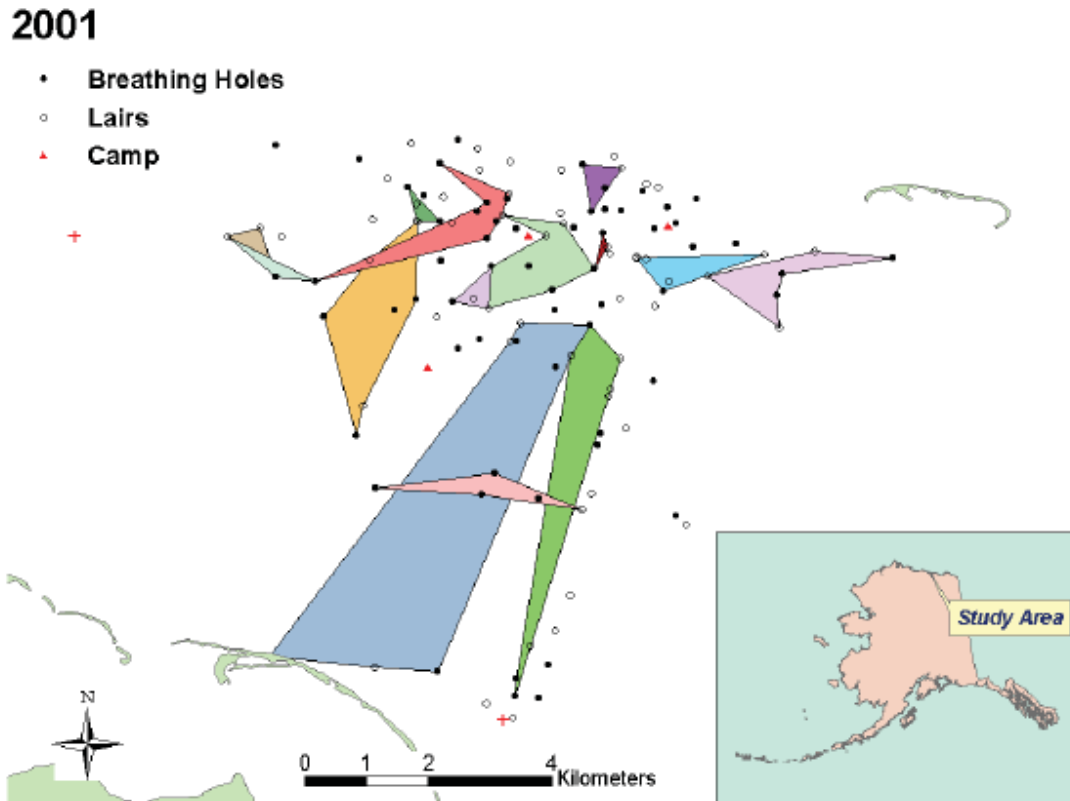


Figure 5. Locations of subnivean breathing holes and lairs located by trained dogs in 2002. The minimum convex polygons delineate the home ranges of 14 ringed seals based on on-ice resting sites. Also shown is the monitoring camp.

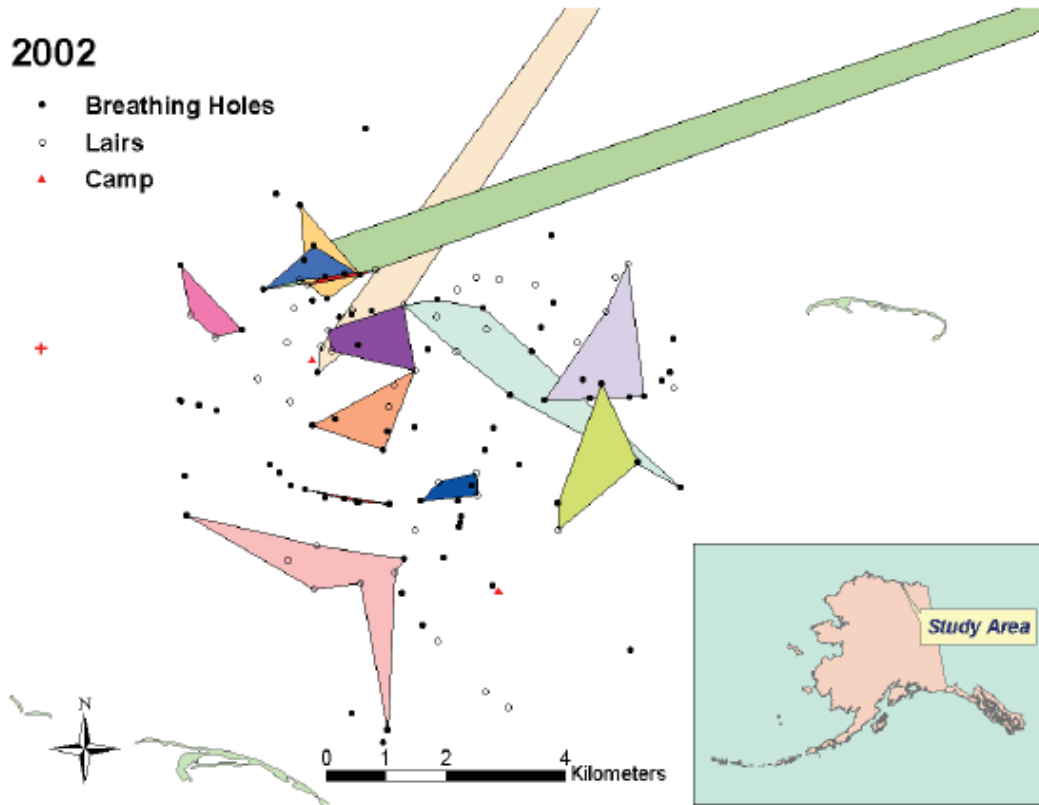


Figure 6. Locations of subnivean breathing holes and lairs located by trained dogs in 2003. The minimum convex polygons delineate the home ranges of 14 ringed seals based on on-ice resting sites. Also shown is the monitoring camp.

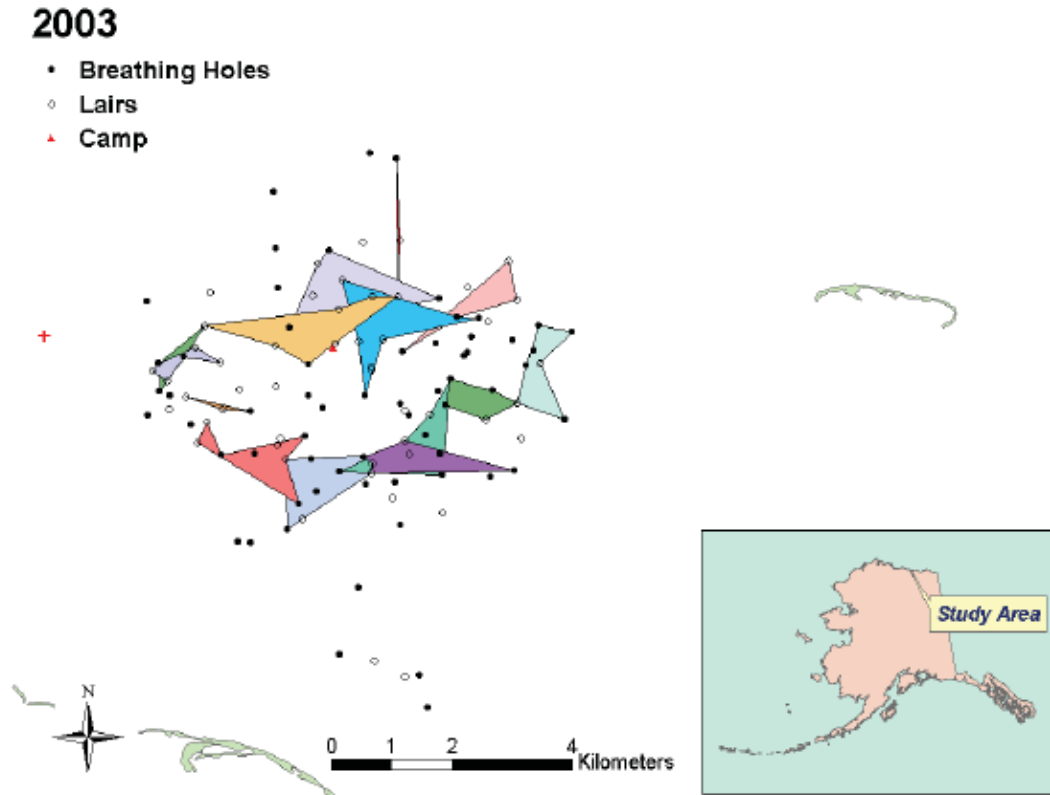
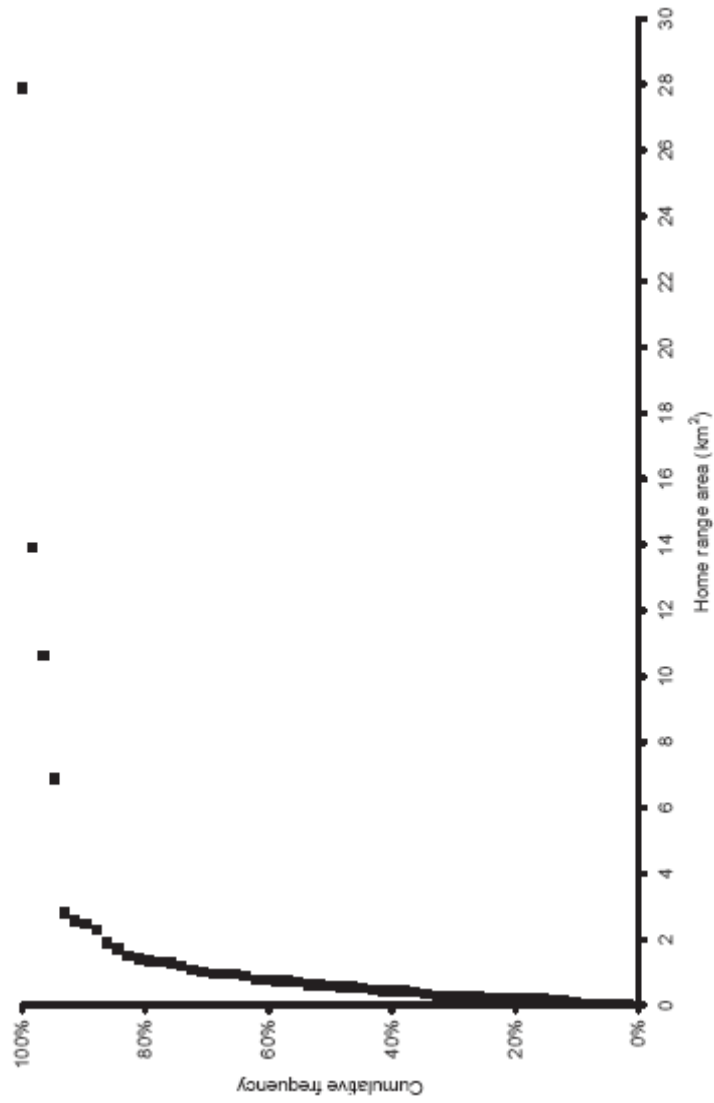


Figure 7. Cumulative percent frequency of home range size (minimum convex polygon) for 58 ringed seals radio tracked during the subnivean period in 1999, 2000, 2001, 2002, and 2003.



recaptured at breathing holes within the same breeding season, 1 to 20 days after their initial capture. Each of those seals was captured within 1300 m of their initial capture site.

Home ranges of different seals overlapped extensively (Figures 2 – 6), and shared use of breathing holes was apparent from our trapping success. We captured two seals at the same breathing hole on 9 occasions, and twice we captured three seals from the same breathing hole.

During the subnivean period, we tracked the three-dimensional movements under the ice of 2 breeding males, 3 breeding females, and 4 subadult seals for periods of 7 to 30 days.

The sub ice volumes used by the seals ranged from 0.03 to 0.13 km³ ($\bar{X} = 0.07$, SD = 0.04) for subadults and breeding males and from 0.10 to 0.17 km³ ($\bar{X} = 0.13$, SD = 0.04) for breeding females. Volumes used by breeding females exceeded those used by breeding males and yearlings primarily in depth. Median dive depths were 89, 106, 137 m for the breeding females, 28 and 35 m for the breeding males, 137 m for a subadult male, and 32, 27, and 64 m for yearlings.

Overlapping under-ice ranges were evident in the four instances where we compared the movements of pairs of seals. In 1990, a yearling female and an adult male were tracked by the same hydrophone array, and their under-ice ranges overlapped by 55.6%. In 1991, the under-ice ranges of a subadult male and a breeding male overlapped by 39.5% and those of two breeding females overlapped by 45.7%. Overlap between a subadult male and a breeding female in 1992 was 55.1%. In each of these cases of overlap, the two seals shared the use of one or more breathing holes.

In April to mid May, tagged seals spent most of their time under the ice. They came out of the water only in subnivean lairs where they spent 16% (SD = 10%) of their time. The duration of subnivean resting bouts ranged from less than 1 to 42 hrs with a median of 6 hrs (Figure 8). The interval between subnivean resting bouts ranged from 1 to 84 hrs with a median of 27 hrs (Figure 9).

Most tagged seals exhibited a significant diel pattern in the proportion of time spent on the ice. Individuals varied in their preferred on-ice times, but subnivean lairs were occupied mostly between 1800 hrs and 0600 hrs (overall $\bar{X} = 0110$ hrs, SD = 6.10 hrs). The variance in times of day during which seals occupied lairs was large (Rao's U = 149.63, $0.10 > p > 0.05$, n = 41), and the probability of an individual seal being out of the water during any hour rarely exceeded 0.50.

Figure 8. Duration (hrs) of haulout bouts in subnivean lairs and out of lairs (basking).

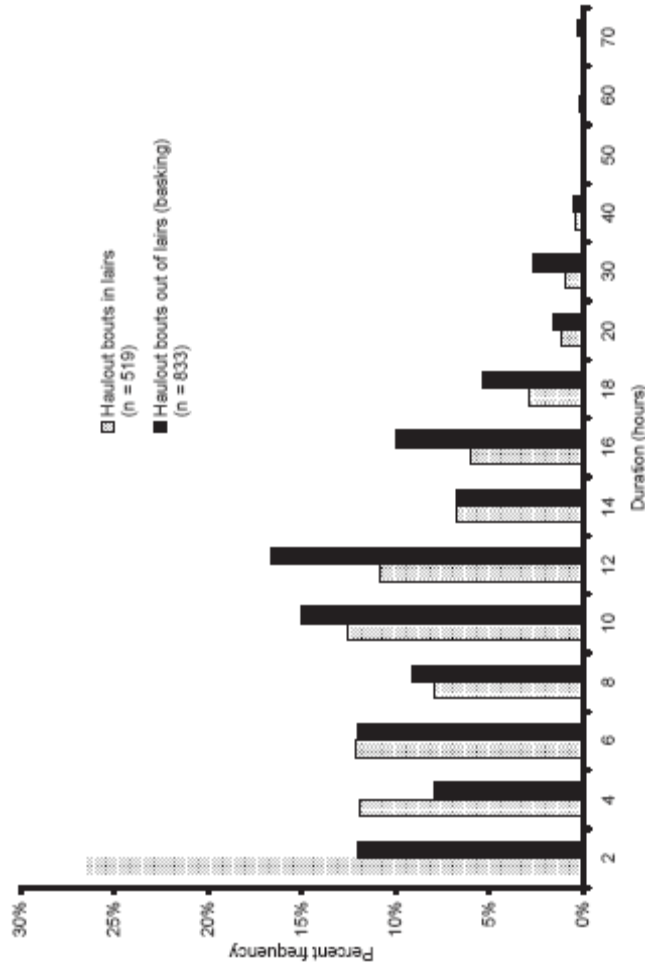
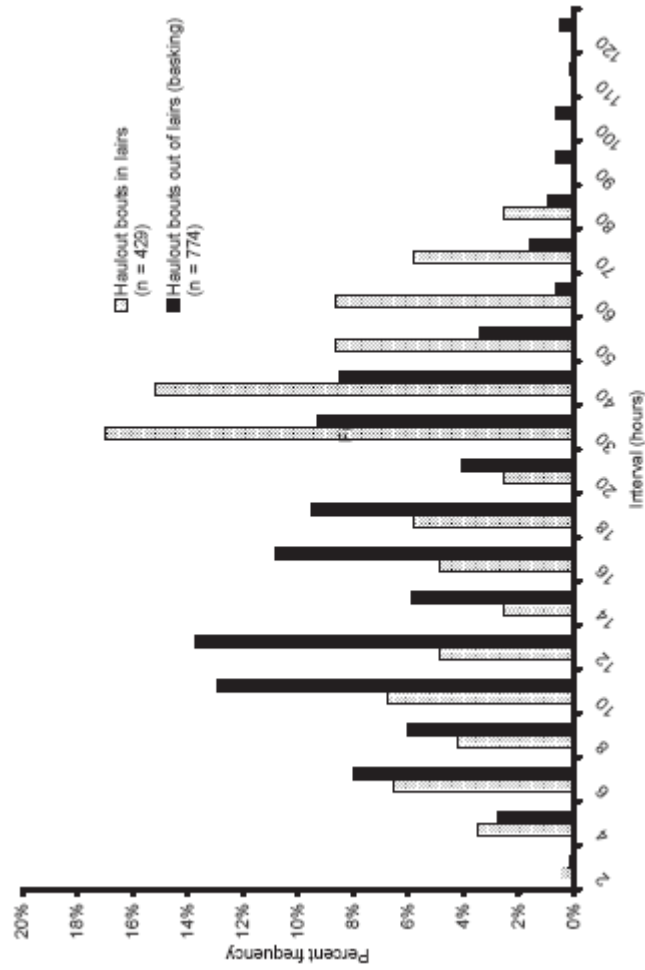


Figure 9. Interval (hrs) between haulout bouts in and out of subnivean lairs.



Basking period

After abandoning their lairs and before ice break up, the radio tagged seals were visible when they basked on the ice. Most (58/60) basked on the ice within the same small home ranges they occupied during the subnivean period. In early June 2002, two seals basked 20 and 40 km, respectively, beyond the ranges they occupied in the subnivean period.

After abandoning their lairs and before ice break up, the amount of time that radio tagged seals spent out of the water increased ($\bar{X} = 55\%$, $SD = 22\%$). Twenty-six of 43 seals monitored before and after their first emergence from lairs occupied lairs one or more times after emergence. They spent an average of 3% (95% CL: 1–4%) of their time in lairs and an average of 37% (95% CL: 32–41%) of their time basking after the first emergence. The median duration of resting bouts on the ice (Figure 8) increased to 9 hrs when the seals were basking while the median intervals between resting bouts (Figure 9) decreased to 14 hrs.

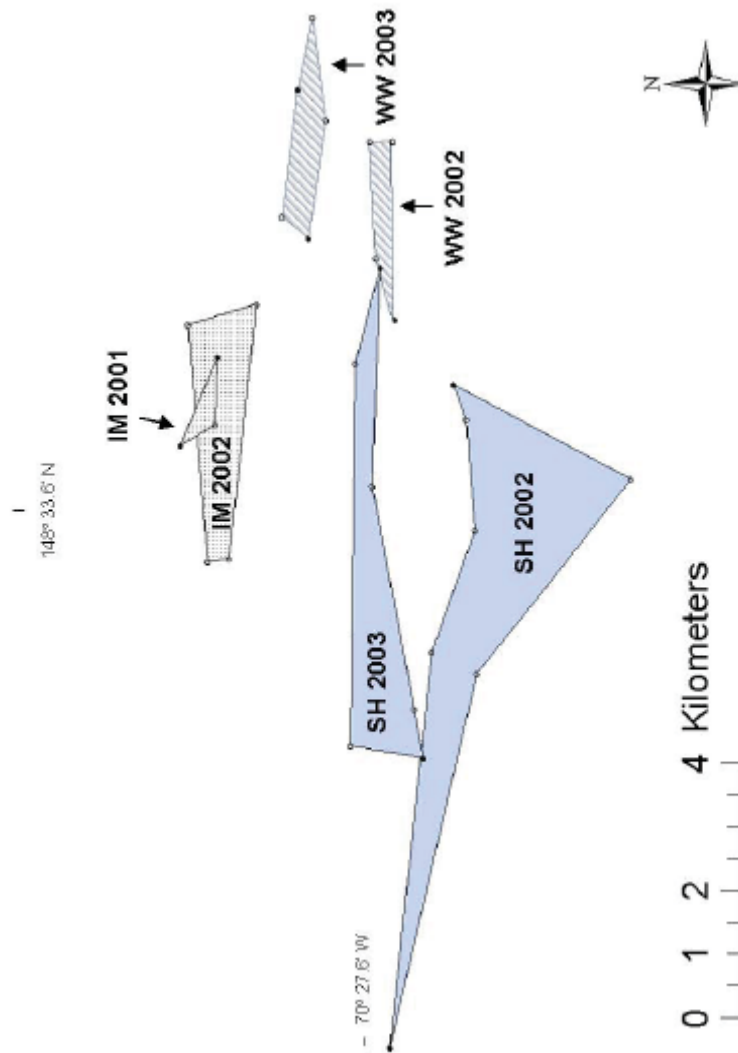
When basking in the spring, the tagged seals were on the ice primarily at mid day ($\bar{X} = 1646$ hrs, $SD = 1.46$ hrs). The shift from being out of the water mostly at night during the subnivean period to being on the ice in late afternoon while basking was highly significant ($U^2 = 1.591$, $p < 0.001$). The variance in time on the ice during the basking period was substantial (Rao's $U = 286.78$, $p < 0.01$, $n = 59$), but the probability of a seal being out of the water during any hour often exceeded 0.50, especially in the afternoon hours.

Three seals tagged with VHF radios during the subnivean period were recaptured in the subsequent subnivean period, 371–377 days after their initial capture. Their recapture sites were 746, 1400, and 2000 m from the sites at which they were captured in the previous year. The home ranges of those three seals were similar in size, configuration, and number of lairs occupied in successive breeding seasons (Figure 10). The unexpected inter annual fidelity of those three seals to the same small home ranges in subsequent breeding seasons prompted us to track the annual movements of additional seals using satellite-linked transmitters.

Foraging period

We tracked the 25 ringed seals carrying satellite-linked transmitters for periods ranging from 4 to 420 days. We received signals from the seven seals tagged north of Inuvik (Beaufort Sea), four of those tagged at Peard Bay (Chukchi Sea), and one of those tagged in Kotzebue Sound (Chukchi

Figure 10. Minimum convex polygons delineating the home ranges of 3 ringed seals each tracked during two successive breeding seasons. IM was an adult male tracked in 2001 and 2002. SH was an adult female tracked in 2002 and 2003. WW was an adult male tracked in 2002 and 2003.



Sea) for less than 100 days. Thus for those 12 seals, we recorded movements during the subnivean and basking periods but not during the foraging period. Four seals (3 tagged in Peard Bay and 1 in Kotzebue Sound) were followed for > 100 and up to 193 days, thus providing movement information during the foraging period but minimally, if at all, during their subsequent subnivean period. Nine seals tagged in Peard Bay were tracked from their tagging dates in May through January or beyond, well in to the subsequent subnivean period. Two of the latter nine seals were tracked to the subsequent June (13 months after tagging) and one to the subsequent July (14 months after tagging).

The 9 seals tracked from one subnivean period to the next, were consistent in their overall pattern of seasonal movements. During the late subnivean period and through the basking periods (May – June) in the year of capture, most seals remained within 50 km of their capture sites (Figure 11). With ice break up in July, they began more extensive movements. From July through early December, movements extended to almost 900 km in the case of one female and over 1700 km in the case of one male. By the subsequent January, 8 of the 9 seals were again close (3 – 54 km) to the sites at which they had been captured during the previous breeding season. The ninth seal, an adult male was not located from November (6 months after capture) until the following May when he was 83 km from the site at which he was captured 12 months earlier. Like the other seals tracked between successive subnivean periods, he made extensive movements during July – September, ranging nearly 1000 km in to the Canadian Beaufort Sea to the east, and then returning to Peard Bay at freeze up in October. In November, however, he made another excursion of almost 800 km across the Chukchi Sea to the coast of Siberia before returning to the Peard Bay region. An adult female similarly ranged over 150 km in July from her breeding site in Peard Bay, returned to within 15 km of her breeding site in October, moved almost 900 km to the north in November, and was back within 3 km of her breeding site in January.

In August through November, the satellite-tracked seals spent 10% or less of their time out of the water (Figure 12). Time out of the water increased slightly in December through March but still was less than 20%. As with the seals tracked by VHF radio, those tracked by satellite-linked transmitters spent less than half of their time out of the water during the subnivean period. In June, however, as most seals were molting, they spent over 60% of their time on the ice. Time out of the water dropped to 30% in July as basking was completed and the foraging period commenced.

While we targeted ringed seals in this study, we did capture and tag two bearded seals in Peard Bay. A yearling male was tracked only for 7 days during which he remained within Peard Bay. A juvenile female was tracked for 378 days and showed a similar movement pattern to that seen in ringed seals. During May and June, she remained within 12 km of her capture site. In July and August, she was north of Pt. Barrow, over 200 km from her capture site. In October, she returned to within 5 km of her Peard Bay capture site and did not range farther than 35 km from the site until the following May. That May (a year after she was tagged) she ranged to 118 km from her original capture site.

Figure 11. Maximal distance from their capture site recorded each month for 9 satellite-tracked ringed seals. Note different scales in top and bottom panels and the break in y-axes on both.

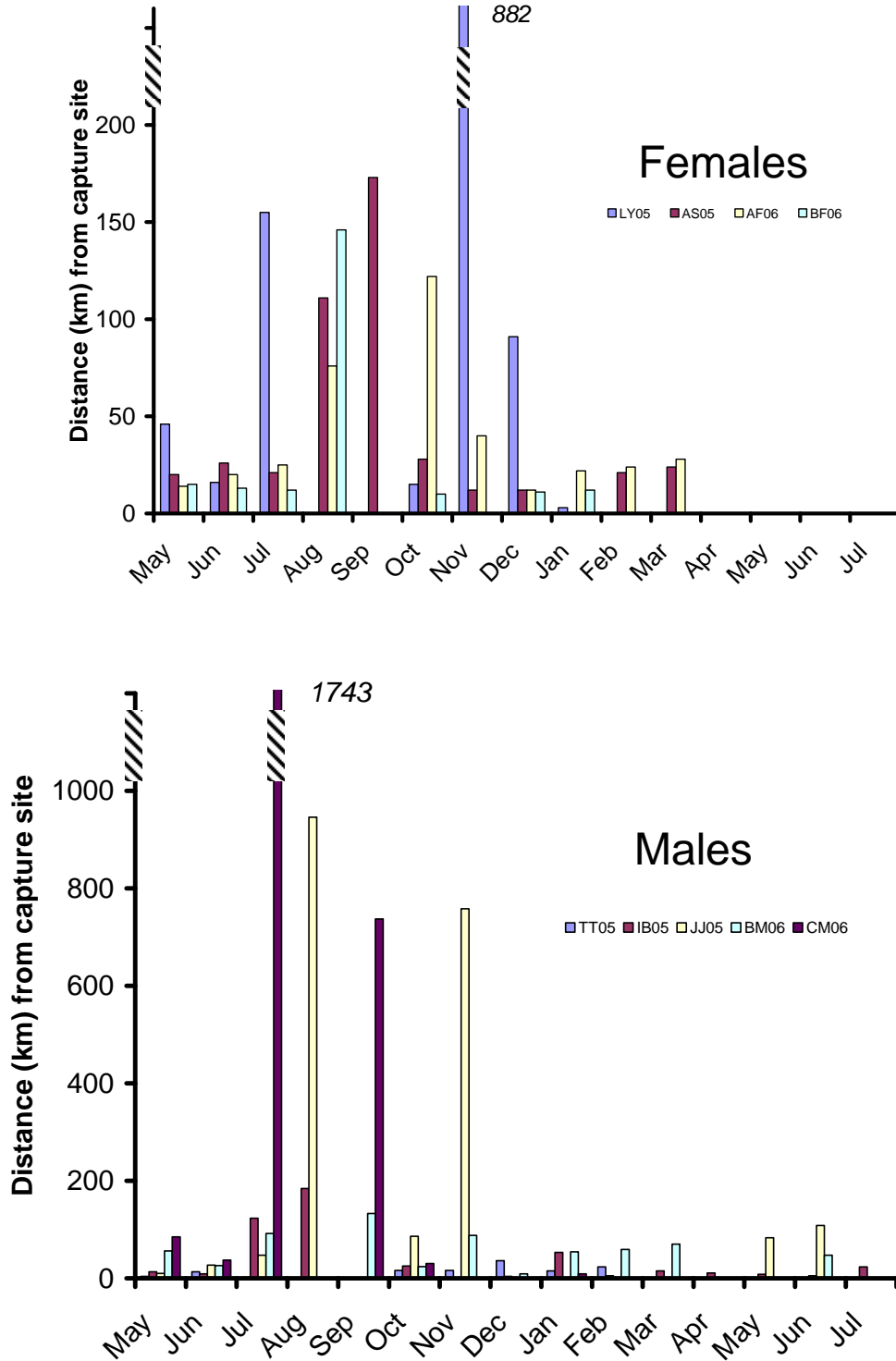
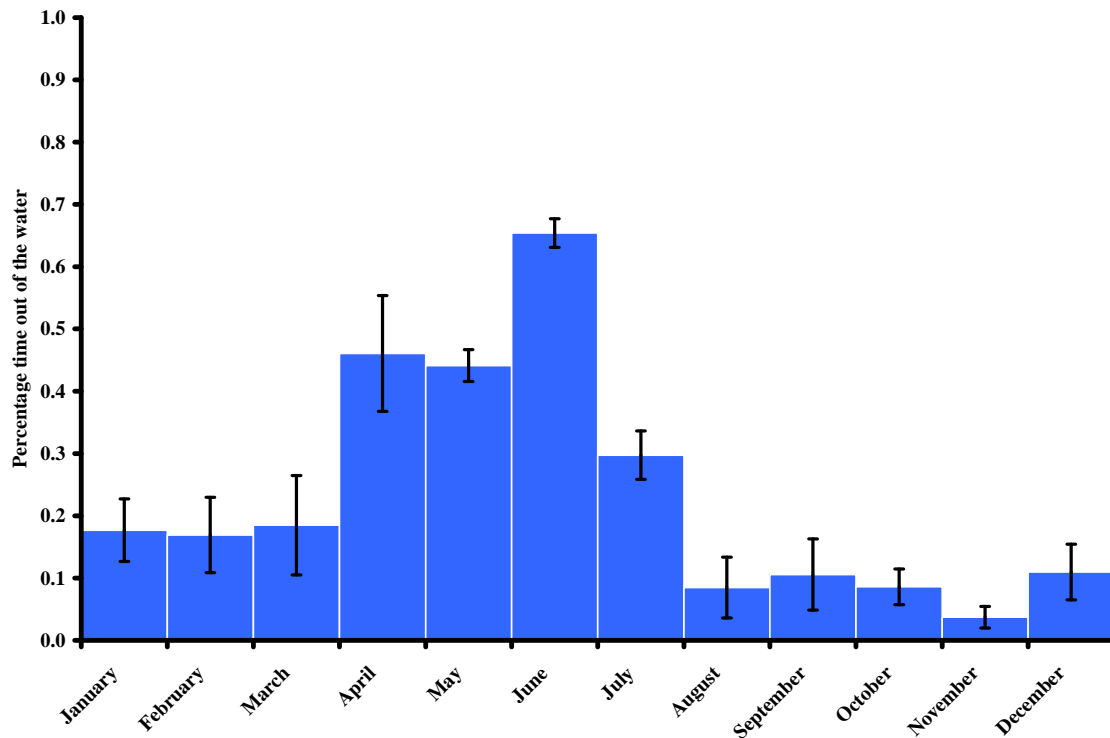


Figure 12. Mean (and 95% confidence limits) time out of the water calculated daily for each of 9 ringed seals tracked by satellite throughout the year.



DISCUSSION

The holes that ringed seals maintain through sea ice provide access to air above and food below. Excavating subnivean lairs above some of those breathing holes permits ringed seals to rest and rear young in an extremely cold environment (Taugbøl 1984; Smith et al. 1991). Adaptations for excavating ice and snow are primarily behavioral but also include stout claws on the foreflippers. The energetic costs of maintaining breathing holes through ice that can exceed two meters in thickness includes restricted home ranges for several months of the year. We found that ringed seals inhabiting shorefast ice in the Beaufort Sea occupied ranges averaging less than 2 km² during April, May, and early June, and the Fall-time movements of the satellite-tagged seals suggested that they probably are restricted to the same ranges from as early as freeze up in September or October.

During the subnivean period, under-ice movements did not substantially exceed the ranges determined by haul-out locations. For periods of 2 to 4 months in summer and fall, when ringed seals forage most intensively, however, they ranged over distances > 100 km and as far as 1800 km, considerably farther than previously documented (Hyvärinen *et al.* 1995, Härkönen et al.

1998, Kunnasranta 2001, Koskela *et al.* 2002). Our movement data likely underestimate the distances travelled during the foraging period, as our tags only reported the seals' locations when out of the water and haulout bouts were rare in the foraging period.

When the shorefast ice forms again in the fall, the seals reoccupy nearly the identical home range used the previous winter and spring. Three seals recaptured at the same breeding site in the Beaufort Sea and 9 seals tracked between successive subnivean periods indicated strong fidelity to breeding sites. Smith and Hammill (1981) observed the same ringed seal resting on the ice of Baffin Island fjord in two successive basking periods. Inter annual fidelity to small ranges has implications for the foraging ecology, breeding biology, and population structure of ringed seals.

During lactation, breeding, and subsequent molting, phocid seals may be restricted in their movements for periods of a few weeks to 3 or 4 months (Costa 1991; Thompson *et al.* 1994; Boyd 1998), although for many species, lactation and foraging patterns are poorly known (Schulz and Bowen 2004). The ringed seals that we tracked were confined to very small ranges for approximately 6-7 months each year. The restriction to small home ranges probably is necessitated by maintenance of breathing holes and appears to limit the range over which ringed seals can forage during most of the year including the lactation period. At least through their first year, young seals, by virtue of their size, are further restricted to feeding in shallow depths (Kelly and Wartzok 1996).

At 6 – 8 weeks, lactation in ringed seals is the longest among the Phocidae (Costa 1991), and their small body size and prolonged confinement to small ranges apparently exceeds the duration of a sustainable fast. Elsewhere, we reported that the ringed seals we tracked under the ice in Resolute Passage made foraging dives throughout the breeding period (Kelly and Wartzok 1996; Simpkins *et al.* 2001). The small under-ice home ranges maintained during the subnivean period allow lactating females to frequently attend the pups in subnivean lairs. Frequent visits to the lairs likely minimize predation on pups (Smith *et al.* 1991) and increase the efficiency of energy transfer to the pup (Boyd 1998). The small size of the under-ice ranges, however, limits access to prey, and ringed seals may compensate by using ranges that correspond to areas of high prey density. We suggest that inter annual fidelity to the same under-ice home ranges reflects selection for occupying areas of high prey density. Foraging apparently intensifies in the few months that ringed seals are not restricted to under-ice home ranges (Lowry *et al.* 1980; Weslawski *et al.* 1994), and during the foraging period, ringed seals range hundreds to thousand of kilometers

from their breeding sites. During the open water period and early part of freeze up, the ringed seals we tracked spent 90% or more of their time in the water. As ice cover thickened in January through March, the time spent in the water remained greater than 80%. Only during the whelping, nursing, and basking periods (April – June) did the time in the water decline to less than 70%.

The selection of breeding habitat by ringed seals has been considered in terms of ice and snow conditions (Chapskii 1940; McLaren 1958; Smith and Stirling 1975; Harding et al. 2001; Kelly et al. 2001; Kelly 2001) and in terms of predation risk (Stirling 1977; Stirling and Archibald 1977; Smith 1976, 1980; Gjertz and Lydersen 1986; Lydersen and Smith 1989; Hammill and Smith 1991; Smith and Lydersen 1991; Smith et al. 1991; Furgal et al. 1996; Kelly 1996; Kelly and Wartzok 1998). Little attention has been directed, however, at the possible influence of prey densities or availability (Nararenko 1967; Siegstad et al. 1998; Holst et al. 2001).

The home ranges we delineated near Prudhoe Bay during the subnivean period in 1999 – 2003 were all in shallow waters (< 10 m) where benthic or epontic organisms may be the only abundant prey. The dynamics of ringed seal prey and their influence on ringed seal home ranges are poorly known, but there is evidence of heterogeneity in benthic production in Arctic seas (Coyle and Highsmith 1994). Benthic and epontic feeding may be especially important when ringed seals inhabit shallow, nearshore waters (such as in our study site near Prudhoe Bay) during the subnivean period. There are patches of highly productive boulders in the near-shore waters of the Alaskan Beaufort Sea (Reimnitz and Ross 1979) that contain diverse macroalgal and invertebrate assemblages (Dunton and Schonberg 2000). Several species of fish also are known to reside in these boulder communities, including the abundant sculpin *Myoxocephalus scorpioides*, whose biomass averages 24 g/m² (Dunton and Schonberg 2000). These rocky outcroppings are potentially rich and valuable sources of food for ringed seals. The under surface of the ice also concentrates ringed seal prey, including gammarid amphipods, in May (Lewis and Milne 1977; Carey 1981) when ringed seals are lactating. Ringed seal feces examined opportunistically in our Resolute Bay and Prudhoe Bay study areas in May revealed the presence of gammarid exoskeletons.

Local concentrations of benthic prey are likely to be stable over time (Bernstein and Meador 1979; Schaffner and Boesch 1982; Carrasco 1997; Mincks *et al.* 2005; Shanks and Eckert 2005) and may favour inter annual fidelity to breeding sites among ringed seals. Concentrations of prey on the under-side of the ice appear to depend, in part, on the topography of that surface (Lewis and Milne 1977; Bradstreet 1982), which likely varies from year to year, and epontic prey

concentrations may be less important in influencing the ringed seal's inter annual fidelity to breeding sites. Our Resolute Passage study site was deeper (225 m) than our other sites, and the seals we tracked there foraged primarily in mid water column in the case of adults or near the under surface of the ice in the case of young animals (Kelly and Wartzok 1996; Simpkins et al. 2001). Site fidelity may offer less advantage in terms of knowledge of local resources in such sites.

Ringed seals in our study sites were still restricted to small under-ice ranges during lactation (April and May) and mating (May and June). Based on the distribution of lairs showing signs of parturition and of breathing holes and lairs smelling of rutting males, Smith and Stirling (1975) and Smith et al. (1994) suggested that males maintained large under-ice territories containing multiple female territories. Our tracking of ringed seals in the Beaufort and Chukchi seas indicated substantial overlap of areas used by breeding females and that the above and under-ice ranges of females were larger than those of males. The male ranges were restricted in depth (Kelly and Wartzok 1996) as well as in the x-y plane. We suggest that neither females nor males exhibit territoriality and that male mating strategy involves a form of mate guarding whereby they station themselves near the primary breathing hole of a post-parturient female until she is receptive. Our tracking data provide little information about male-male interactions, but we observed substantial overlap in the under-ice ranges of all age classes. We also observed - as have others (Smith et al. 1994) - an increase in fresh wounds to the hindfippers of males with the advent of breeding. The substantial sharing of breathing holes that we observed included many instances of breeding males sharing holes with adult females. We also detected the strong odour of rutting males at birth lairs, indicating that one or more males had visited a lair used by a female and her pup.

It may well be that patterns of social interaction vary over the wide range of ringed seals. For example, in Lake Ladoga, Russia, multiple non-breeding ringed seals occupy the same lair (Kunnasranta *et al.* 2001). In Lake Baikal, however, ringed seal home ranges (measured at the surface) did not overlap and seals are reported to avoid each other during the basking period (Martinkova *et al.* 2001). Our sample of seals in shorefast ice in Alaska and Canada may not be representative of seals occupying pack ice or even shorefast ice in other parts of the Arctic.

While the concentration of estrus females in space and time has led to high levels of polygyny in many pinniped species (Batholomew 1970; Emlen and Orring 1977), aquatic, under-ice breeding among ringed seals appears to reduce the potential for polygyny. We suggest that the necessity of

maintaining breathing holes constrains ringed seals to low levels of polygyny through serial monogamy.

Our tracking records provide strong evidence of inter annual fidelity to breeding sites among ringed seals. Our single record of inter annual movements by a bearded seal suggests site fidelity in that species as well. Site fidelity has been documented in several other phocid species including harbor seals (*Phoca vitulina*), grey seals (*Halichoerus grypus*), southern elephant seals (*Mirounga leonina*), and Weddell seals (*Leptonychotes weddellii*) (Allen *et al.* 1995; Goodman 1998; Pomeroy *et al.* 2000; Fabiani *et al.* 2006; Cameron *et al.* 2007; Poland *et al.* 2008). Fidelity to breeding sites suggests that gene flow may be restricted. Our tracking data for ringed and bearded seals are limited, however, to observations of individuals in two successive breeding seasons, at best, and whether the observed site fidelity reflects natal philopatry remains to be determined. We have initiated an investigation of molecular markers from around the Arctic to further investigate population structuring among ringed seals.

We expect the importance of understanding the populations structure of ice-associated seals to increase as their sea ice habitat diminishes. In recent centuries, Northern Hemisphere sea ice provided over 16,000,000 km² of substrate on which ice-associated seals rested, gave birth, nursed their young, and completed an annual molt. In recent decades, however, that substrate has been decreasing (Overland and Wang 2007; Serreze *et al.* 2007; Comiso *et al.* 2008), and summer ice may disappear completely within the next 50 years. That reduction in sea ice will negatively impact ringed seal populations (Tynan and DeMaster 1997; Kelly 2001; Smith and Harwood 2001; Ferguson *et al.* 2005).

Changes in community structure associated with diminished sea ice will affect food sources for ringed seals. For example, shorter periods of ice cover will decrease the supply of ice algae, and increase the supply of phytoplankton, reaching benthic shelf communities (Overland and Stabeno 2004; Grebmeier *et al.* 2006). Arctic cod, an important prey of ringed seals, rely on sea ice as cover from their many predators (Bradstreet and Cross 1982; Poltermann 2001), and the fate of these fish is uncertain.

At the same time, increasingly early snow melts will further threaten ringed seal recruitment (Kelly 2001; Smith and Stirling 2002). The vulnerability of ringed seals to losses of snow and ice cover will be determined, in large measure, by population structure. If, as suggested by the

observed fidelity to breeding sites, they are comprised of many locally adapted demes, the potential for local extinction is much higher than if there is extensive gene flow.

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LITERATURE CITED

- Allen, P. J., W. Amos, P. P. Pomeroy, and S. D. Twiss. 1995. Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Molecular Ecology* 4(6):653-662.
- Barber, C. B., D. P. Dobkin, and H. T. Huhdanpaa. 1996. The Quickhull algorithm for convex hulls. *ACM Transactions on Mathematical Software*, 22:469-483. <http://www.qhull.org>.
- Bartholomew, G. A. 1970. A model for the evolution of pinniped polygyny. *Evolution* 24:546-559.

- Bernstein, B. B., and J. P. Meador. 1979. Temporal persistence of biological patch structure in abyssal benthic community. *Marine Biology* 51(2):179-183.
- Boas, F. 1888. The Central Eskimo. *Ann. Rep. Bur. Am. Ethnol.* 6:339-669. Smithsonian Institution, Washington, D.C.
- Boyd, I. L. 1998. Time and energy constraints in pinniped lactation. *The American Naturalist* 152:717-728.
- Bradstreet, M. S. W. 1982. Occurrence, habitat use, and behavior of seabirds, marine mammals, and Arctic cod at the Pond Inlet ice edge. *Arctic* 35:28-40.
- Bradstreet, M.S.W. and W.E. Cross. 1982. Trophic relationships at high arctic ice edges. *Arctic* 35:1-12.
- Burns, J. J. 1970. Remarks on the distribution and natural history of pagophilic pinnipeds in the Bering and Chukchi Seas. *J. Mammalogy* 51:445-454.
- Cameron, M. F., D. B. Siniff, K. M. Proffitt, and R. A. Garrott. 2007. Site fidelity of Weddell seals: the effects of sex and age. *Antarctic Science* 19(2):149-155.
- Carrasco, F. D. 1997. Sublittoral macrobenthic fauna off Punta Coloso, Antofagasta, northern Chile: high persistence of the polychaete assemblage. *Bulletin of Marine Science* 60:443-459.
- Chapskii, K. K. 1940. The ringed seal of western seas of the Soviet Arctic (The morphological characteristic, biology and hunting production). *Tr. Vses. Arkt. Inst. (Leningrad)* 145:1-72. (Transl. from Russian by Fish. Res. Board Can., 1971, Transl. Ser. 1665, 147pp.).
- Costa, D. P. 1991. Reproductive and foraging energetics of pinnipeds: implications for life history patterns. Pages 300-344 *in* D. Renouf, editor. *The behaviour of pinnipeds*. Chapman & Hall, London.
- Cox, S. L. and A. Spiess. 1980. Dorset settlement and subsistence in northern Labrador. *Arctic* 33:659-669.
- Coyle, K. O., and R. C. Highsmith. 1994. Benthic amphipod community in the northern Bering Sea: analysis of potential structuring mechanisms. *Mar. Ecol. Prog. Ser.* 107: 233-244.

- Davies, J. L. 1958. The Pinnipedia: an essay in zoogeography. *Geographical Review* 48:474-493
- Davis, S. C. 2004. Phylogenetic relationships of the Phocidae and population genetics of ice-breeding seals. PhD Thesis, University of Alberta, Edmonton, Alberta.
- Dunton, K.H. and S.V. Schonberg. 2000. The benthic faunal assemblage of the Boulder Patch kelp community, Chapter 18. *In: The Natural History of an Arctic Oil Field*. Academic Press.
- Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215-223.
- Fabiani, A., F. Galimberti, S. Sanvito, and R. Hoelzel. 2006. Relatedness and site fidelity at the southern elephant seal, *Mirounga leonina*, breeding colony in the Falkland Islands. *Animal Behaviour* 72:617-626.
- Feltz, E.T., and F.H. Fay. 1966. Thermal requirements in vitro of epidermal cells from seals. *Cryobiology* 3:261-264.
- Ferguson, S. H., I. Stirling, and P. Mcloughlin. 2005. Climate change and ringed seal (*Phoca hispida*) recruitment in Western Hudson Bay. *Marine Mammal Science* 21:121-135.
- Gjertz, I., K. M. Kovacs, C. Lydersen, and O. Wiig. 2000. Movements and diving of adult ringed seals (*Phoca hispida*) in Svalbard. *Polar Biology*. 23: 651-656.
- Goodman, S. J. 1998. Patterns of extensive genetic differentiation and variation among European harbor seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorphisms. *Mol. Biol. Evol.* 15(2):104-118.
- Grebmeier, J. M., J. E. Overland, S. E. Moore, E. V. Farley, E. C. Carmack, L. W. Cooper, K. E. Frey, J. H. Helle, F. A. McLaughlin, and S. L. McNutt. 2006. A major ecosystem shift in the northern Bering Sea
- Hall, C. F. 1866. Arctic researches and life among the Esquimaux: being the narrative of an expedition in search of Sir John Franklin, in the years 1860, 1861, and 1862. Harper Brothers Publishers, New York.

- Härkönen, T., O. Stenman, M. Jüssi, I. Jüssi, R. Sagitov, and M. Verevkin. 1998. Population size and distribution of the Baltic ringed seal (*Phoca hispida botnica*). In M. P. Heide-Jørgensen, and C. LYDERSEN (eds.) Ringed seals in the North Atlantic pp.167-180.
- Heide-Jørgensen, M. P., B. S. Stewart, and S. Leatherwood. 1992. Satellite tracking of ringed seals *Phoca hispida* off northwest Greenland. *Ecography* 15:56-61.
- Hyvärinen, H., E. Hämäläinen, and M. Kunnasranta. 1995. Diving behavior of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.). *Marine Mammal Science* 11:324-334.
- Kapel, F. O., J. Christiansen, M. P. Heide-Jørgensen, T. Härkönen, E. W. Born, L. Knutsen, F. Riget, and J. Teilmann. 1998. Netting and conventional tagging used to study movements of ringed seals (*Phoca hispida*) in Greenland. Pages 211-228 in M. P. Heide-Jørgensen and C. Lydersen (eds.) Ringed seals in the North Atlantic. Volume 1. North Atlantic Marine Mammal Commission, Tromsø.
- Kelly, B. P. 1988. Ringed seal, *Phoca hispida*. Pages 59-75 in J. W. Lentfer, ed. Selected marine mammals of Alaska: species accounts with research and management recommendations. Marine Mammal Commission, Washington, D.C.
- Kelly, B. P. 1996. Live capture of ringed seals in ice-covered waters. *Journal of Wildlife Management* 60: 678-684.
- Kelly, B. P. 2001. Climate change and ice breeding pinnipeds. In: "Fingerprints" of climate change: adapted behaviour and shifting species' ranges. G.-R. Walther, C. A. Burga, P. J. Edwards (eds.) Kluwer Academic/Plenum Publishers, New York and London, 43-55.
- Kelly, B.P. and L.T. Quakenbush. 1987. Trained dogs and wild seals. *Whalewatcher, J. Amer. Cetacean Soc.* 21:8-11.
- Kelly, B.P., and L. T. Quakenbush. 1990. Spatiotemporal use of lairs by ringed seals (*Phoca hispida*). *Canadian Journal of Zoology*.
- Kelly, B. P. and D. Wartzok. 1996. Ringed seal diving behavior in the breeding season. *Canadian journal of Zoology* 74: 1547- 1555

- King, J. E. 1983. Seals of the world, 2nd Edition. Comstock Publishing Associates, Ithaca, NY.
- Koskela, J. T., M. Kunnasranta, E. Hämäläinen, and H. Hyvärinen. 2002. Movements and use of haul-out sites of radio-tagged Saimaa ringed seal (*Phoca hispida saimensis* Nordq.) during the open-water season. *Annales Zoologica Fennici* 39: 59-67.
- Kunnasranta, M. 2001. Behavioural biology of two ringed seal (*Phoca hispida*) subspecies in the large European lakes Saimaa and Ladoga. PhD thesis, University of Joensuu.
- Kunnasranta, M., H. Hyvärinen, T. Sipilä, and N. Medvedev. 2001. Breeding habitat and lair structure of the ringed seal (*Phoca hispida ladogensis*) in northern Lake Ladoga in Russia. *Polar Biology* 24:171-174.
- Lowry, L. F., K. J. Frost, and J. J. Burns. 1980. Variability in the diet of ringed seals, *Phoca hispida*, in Alaska. *Can. J. Fish. Aquat. Sci.* 37:2254-2261.
- Martinkova, N., A. Zahradnikova, J. A. Budeev, and P. Vransky. 2001. Surface home ranges of the Baikal seal (*Phoca sibirica*) during the solid ice-cover period. *Biologia, Bratislava* 56:219-224.
- McLaren, I.A. 1958. The biology of the ringed seal (*Phoca hispida* Schreber) in the eastern Canadian arctic. Fisheries Research Board of Canada. Ms. Rep. (Biology) 653. 146 pp.
- Mincks, S. L., C. R. Smith, and D. J. DeMaster. 2005. Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: evidence of a sediment 'food bank.' *Marine Ecology Progress Series* 300:3-19.
- Overland, J. E., P. J. Stabeno. 2004. Is the climate of the Bering Sea warming and affecting the ecosystem? *EOS* 85:309-312.
- Poland, V. F., P. P. Pomeroy, S. D Twiss, and J. A Graves. 2008. Fine-scale study finds limited evidence of kin clustering in a gray seal colony. *Marine Mammal Science* 24(2):371-387.
- Poltermann, M. 2001. Arctic sea ice as feeding ground for amphipods – food sources and strategies. *Polar Biol.* 24:89-96.
- Pomery, P. P., S. D. Twiss, and P. Redman. 2000. Philopatry, site fidelity and local kin associations within grey seal breeding colonies. *Ethology* 106:899-919.

- Reeves, R. R. 1988. Distribution, abundance and biology of ringed seals (*Phoca hispida*): an overview. Pages 9 – 45 in M. P. Heide-Jorgensen, and C. Lydersen (eds.) Ringed seals in the North Atlantic.
- Ridoux, V., A. J. Hall, G. Steingrímsson, and G. Olafsson. 1998. An inadvertent homing experiment with a young ringed seal, *Phoca hispida*. Mar. Mamm. Sci. 14:883-888.
- Sabo III, G. and J. D. Jacobs. 1980. Aspects of Thule culture adaptations in southern Baffin Island. Arctic 33:487-504.
- Schaffner, L. C., and D. F. Boesch. 1982. Spatial and temporal resource use by dominant benthic Amphipoda (Ampeliscidae and Corophiidae) on the Middle Atlantic Bight Outer Continental Shelf. Marine Ecology Progress Series 9:231-243.
- Scheffer, V. B. 1958. Seals, sea lions and walruses. A review of the pinnipedia. Stanford University Press, Stanford, Calif.
- Schultz, T. M., and W. D. Bowen. 2004. Pinniped lactation strategies: evaluation of data on maternal and offspring life history traits.
- Shanks, A. L., and G. L. Eckert. 2005. Population persistence of California current fishes and benthic crustaceans: a marine drift paradox. Ecological Monographs 75(4):505-524.
- Simpkins, M. A., B. P. Kelly, and D. Wartzok. 2001. Three-dimensional analysis of search behaviour by ringed seals. Animal Behaviour 62:67-72.
- Smith, T.G. 1987. The ringed seal, *Phoca hispida*, of the Canadian western Arctic. Can. Bull. Fish. and Aquat. Sci. 216. Ottawa. 81pp.
- Smith, T. G. and M. O. Hammill. 1981. Ecology of the ringed seal, *Phoca hispida*, in its fast ice breeding habitat. Canadian Journal of Zoology. 59: 966-98.
- Smith, T. G., M. O. Hammill, and G. Taugbøl. 1991. A review of the developmental, behavioural and physiological adaptations of the ringed seal, *Phoca hispida*, to life in the Arctic winter. Arctic 44:124-131.

- Smith, T. G., and L. A. Harwood. 2001. Observations of neonate ringed seals, *Phoca hispida*, after early break-up of the sea ice in Prince Albert Sound, Northwest Territories, Canada, spring 1998. *Polar Biol.* 24:215-219.
- Smith, T.G., and I. Stirling. 1975. The breeding habitat of the ringed seal (*Phoca hispida*); The birth lair and associated structures. *Can. J. Zool.* 53:1297-1305.
- Stirling, I., and T. G. Smith. 2004. Implications of warm temperatures and an unusual rain event for the survival of ringed seals on the coast of Southeastern Baffin Island. *Arctic* 57:59-67.
- Taugbøl, G. 1984. Ringed seal thermoregulation, energy balance and development in early life, a study on *Pusa hispida* in Kongsfj., Svalbard. Thesis, Zoofysiologisk Institutt, University of Oslo, Norway. 1982. 102pp. (Canadian translation of Fisheries and Aquatic Sciences No. 5090).
- Taylor, W. E. 1968. An archaeological overview of Eskimo economy. In V. F. Valentine and F. G. Vallee (eds.) *Eskimo of the Canadian Arctic*. D. Van Nostrand Co., Princeton.
- Teilmann, J., E. W. Born, and M. Acquarone. 1999. Behaviour of ringed seals tagged with satellite transmitters in the North Water polynya during fast-ice formation. *Canadian Journal of Zoology* 77:1934- 1946.
- Thompson, P. M., D. Miller, R. Cooper, and P. S. Hammond. 1994. Changes in the distribution and activity of female harbour seals during the breeding season: implications for their lactation strategy and mating patterns. *Animal Ecology* 63:24-30.
- Tynan, C. T., and D. P. DeMaster. 1997. Observations and predictions of arctic climatic change: potential effects on marine mammals. *Arctic* 50:308-322.
- Wartzok, D., S. Sayegh, H. Stone, J. Barchak, and W. Barnes. 1992. Acoustic tracking system for monitoring under-ice movements of polar seals. *J. Acoustical Society of America* 92:682-687.
- Weslawski, J. M., M. Ryg, T. G. Smith, and N. A. Oritsland. 1994. Diet of ringed seals (*Phoca hispida*) in a fjord of West Svalbard. *Arctic* 47:109-114.

Worton, B. J. 1987. A review of models of home range for animal movement. *Ecol. Model.* 38:277-298.

Chapter 2 - Shed skin as a source of DNA for genotyping seals

Publication in *Molecular Ecology Notes*

TECHNICAL NOTE

Shed skin as a source of DNA for genotyping seals

B. J. SWANSON,* B. P. KELLY,† C. K. MADDOX* and J. R. MORAN†

*

Applied Technology in Conservation Genetics Laboratory, Brooks Hall, Central Michigan University, Mount Pleasant, MI 48859, USA,

†

School of Arts and Sciences, University of Alaska Southeast, Juneau, AK 99801, USA

Abstract

Obtaining a sufficient number of DNA samples from ice-breeding marine phocids, in a noninvasive manner, has proven difficult and has limited the ability to use molecular genetics on these species. We evaluate the ability to genotype ringed seals using a novel source of DNA, skin cells shed by the seal as it moults on sea ice. We found that shed skin samples yielded a lower quantity and purity of DNA compared to tissue samples. Nevertheless, the shed skin cells were a viable source of DNA for microsatellite analysis; we found no significant difference in allelic diversity or heterozygosities between tissue samples and shed skin cells. This source of DNA should allow the rapid collection of a large number of noninvasively collected DNA samples in ice-breeding phocids.

Keywords: Arctic, genotyping, microsatellites, noninvasive, *Phocidae*, seals

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The introduction of polymerase chain reaction (PCR) prompted a rapid expansion in the types of samples from which analyzable quantities of DNA can be obtained (Pääbo 1990), often called low copy DNA (lcDNA) sources (e.g. Gerloff *et al.* 1995; Ernest *et al.* 2002; Lucchini *et al.* 2002; Palomares *et al.* 2002). The ability to obtain usable quantities of DNA from sources such as faeces (e.g. Høss *et al.* 1992; Maudet *et al.* 2004), blood (e.g. Dallas *et al.* 2003; Scandura 2005), hair (e.g. Goossens *et al.* 2000; Constable *et al.* 2001), urine (e.g. Hedmark *et al.* 2004), feathers (e.g. Rudnick *et al.* 2005) and scales (e.g. Miller & Kapuscinski 1997; Säisä *et al.* 2003) has also revolutionized data collection methods for biologists. Non-invasive DNA collection is now

commonplace, reducing danger and stress to both animals and biologists and increasing the ability to obtain sample sizes necessary for accurate parameter estimation (Taberlet *et al.* 1997; Creel *et al.* 2003).

The increasing frequency of lcDNA use also led to the recognition of several problems associated with these sources such as allelic dropout or misprinting; errors which can occur at high frequencies (Creel *et al.* 2003). Allelic dropout and misprinting are associated with lcDNA due to the low number of cells found in most samples and the extensive exposure to environmental conditions often associated with the collection of lcDNA. Low-copy DNA samples typically are removed from the environment after exposure to ambient conditions for hours to days or even longer. Depending upon the season, the samples can be exposed to intense periods of UV radiation, temperature fluctuations and precipitation, resulting in degraded DNA. Several approaches to reducing the impact of these errors have been developed (e.g. Taberlet *et al.* 1996; Morin *et al.* 2001; Miller *et al.* 2002; Creel *et al.* 2003; Bellemain & Taberlet 2004).

Perhaps the most valuable impact of noninvasive sampling has been the increased ability to sample rare or elusive species (Taberlet *et al.* 1997; Ernest *et al.* 2000; Hedmark *et al.* 2004; Swanson & Rusz 2006). These techniques, however, have been of limited utility in sampling marine mammals, even if the species of interest spend some portion of their time on land or ice (but see Reed *et al.* 1997; Parsons *et al.* 1999; Parsons 2001).

Most species of seals spend the majority of their time in the ocean, coming out of the water to molt, rest, give birth and nurse their young. Sampling ice dwelling mammals, such as ringed seals (*Phoca hispida*), can be even more difficult as they rarely are found far from accessible water. Nursing phocids rapidly put on weight minimizing the amount of time they, and their mothers, spend on the ice (Perry *et al.* 1995). Thus, obtaining a sufficient number of samples from marine mammals in a nondestructive and noninvasive manner is problematic.

In May 2005, shed skin samples were collected from the ice around ringed seal breathing holes in the shorefast ice of the Chukchi Sea. Subnivean breathing holes were located by Labrador retriever dogs trained to dig in the snow where they detected the odour of ringed seals (Smith & Stirling 1975; Kelly & Quakenbush 1987). Other sample sites were located by visually scanning the ice for seals resting on the surface of the ice. Additional skin samples were collected from ringed seals captured at their breathing holes using the methods described by Kelly (1996). A 5

mm × 5 mm skin sample was clipped from the webbing of the hind flipper of each captured seal, placed in a coin envelope, and stored dry at approximately 10 °C.

Individual flakes of shed skin were collected from the surface of the ice next to breathing holes using sterilized forceps, placed in to a coin envelope, and stored dry. The location of each hole was recorded using hand-held GPS receiver along with the date, time and a description of each site including evidence that more than one seal had been present.

Extractions were performed with QIAGEN DNeasy kits following published protocols (QIAGEN 2001) and DNA quantity and purity were measured with an Eppendorf BioPhotometer (Brinkman Instruments Inc.) using single use uvets (Fisher Scientific). Samples were diluted to a working stock of 15 ng/μL, and any remaining tissue and extracted DNA were stored at -80°C. All seals were genotyped at six microsatellite loci developed for harbour seals (Allen *et al.* 1995; Goodman 1998). All reverse primers were fluorescently labeled with FAM, TET, or HEX. PCR was performed in a 20-μL cocktail containing 75 ng genomic DNA, 250 μM dNTPs, 0.16 μM of each primer, 1 × HotMaster *Taq* buffer, and 1.5 U of HotMaster *Taq* polymerase (Brinkman Instruments Inc.). Amplification was conducted on an Eppendorf MasterGradient Thermocycler (Brinkman Instruments Inc.) and consisted of initial denaturation step for 2 min at 94 °C followed by three cycles of 20 s at 94°C, 20 s at 52°C, and 5 s at 72°C. This was followed by 33 cycles of 15 s at 94°C, 20 s at 52°C, and 5 s at 72°C, followed by a terminal extension step of 1 min at 72°C. Following amplification, samples were analyzed on an ABI PRISM 310 Genetic Analyzer using genescan analysis 3.1.2 software (Applied Biosystems). Genotypes were determined using genotyper 2.0 software (Applied Biosystems).

All shed skin samples, which produced a homozygous genotype, were re-run in two additional PCRs to minimize the possibility of allelic dropout. In cases where any of the three PCRs indicated a heterozygote existed, the individual was classified as a heterozygote.

We found that the tissue samples (mean = 105.6 ng/μL, SE = 8.0) had a significantly higher DNA concentration ($t_{df=97} = 8.2$, $P << 0.0001$) than the shed skin samples (mean = 29.4 ng/μL, SE = 4.8). The tissue samples (mean = 1.6, SE = 0.02) also had a significantly higher ($t_{df=97} = 10.0$, $P << 0.0001$) 260/280 absorbance value than the shed skin samples (mean = 1.1, SE = 0.05). A pure DNA preparation should produce a 260/280 purity value of 1.8 (Manchester 1995).

We were unable to achieve any amplification for 5 (15.6%) of our shed skin samples. It was possible that a shed skin sample could contain DNA from more than one individual given our collection methods; we classified a sample as containing multiple individuals if any locus showed more than two alleles in any amplification at any locus. We found evidence of multiple seals within a sample in nine of the 27 samples (33%). Notably, eight of these samples were identified in the field as likely containing multiple seals. All of the samples containing multiple individuals were removed from all further analyses. Genepop indicated that none of the loci in either the tissue or the shed skin samples were out of Hardy–Weinberg equilibrium (all $P > 0.18$) and none of the loci showed linkage disequilibrium (all $P > 0.22$). Allelic drop out was rare in the shed skin samples and was found in only five of 216 (2.3%) amplifications.

We found high levels of heterozygosities at all loci (0.59–0.88; Table 1) and a large number of alleles at all loci (11–19; Table 1). We found no significant difference (all $P > 0.27$) in the average heterozygosities at any of the loci based on sample type following 1000 resamplings to equalize sample size (Table 1). We found the same pattern with respect to allelic diversity based on 1000 resamplings; there were no significant differences (all $P > 0.17$) in the number of alleles at any of the six loci based on the source of the DNA (Table 1).

Table 1 Observed heterozygosity (H_O) and the number of alleles (A) found at each locus. Samples came from either live-trapped seals (tissue) or from skin cells collected around breathing holes (skin). P value refer to the comparison between heterozygosity values and allelic diversity values for the skin samples vs. the tissue samples. All P values are based on 1000 resamplings of the data for the tissue samples down to the sample size for the skin samples

	SGPV9		SGPV10		SGPV16		Hg4.2		Hg6.3		Hg8.10	
	H_O	A	H_O	A	H_O	A	H_O	A	H_O	A	H_O	A
Total	0.67	11	0.87	17	0.83	11	0.84	18	0.88	17	0.51	15
Skin ($n = 18$)	0.56	7	0.80	13	0.78	7	0.81	10	0.94	10	0.56	8
Tissue ($n = 51$)	0.69	9	0.90	16	0.83	11	0.89	17	0.87	17	0.67	14
P value	0.27	0.84	0.36	0.78	0.33	0.62	0.41	0.20	0.83	0.17	0.58	0.19

Despite the harsh conditions the shed skin cells were exposed to while on the ice, they still provided a valuable and reliable source of DNA. All of our noninvasively collected samples produced either complete genotypes or no amplification, and we found a very low level of allelic dropout. We conclude that shed skin samples can be used to accurately characterize an individual. Similarly, the skin cells shed on the ice next to the seals' breathing holes had the same average

heterozygosity and allelic diversity as the DNA from more traditional sources indicating that they can be used to characterize a population as well.

The ability to use shed skin cells from marine mammals will improve the ability of researchers to obtain a large number of genetic samples on species which, previously, were difficult to sample in sufficient quantity. This should positively impact biologists' ability to investigate the breeding structure of pinnipeds and improve our ability to manage these species as well.

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References

- Allen PJ, Amos W, Pomeroy PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Molecular Ecology*, **4**, 653–662.
- Bellemain E, Taberlet P (2004) Improved noninvasive genotyping method: application to brown bear (*Ursus arctos*) faeces. *Molecular Ecology Notes*, **4**, 519–522.
- Constable JL, Ashley MV, Goodall J, Pusey AE (2001) Noninvasive paternity assignment in Gombe chimpanzees. *Molecular Ecology*, **10**, 1279–1300.
- Creel S, Spong G, Sands JL *et al.* (2003) Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. *Molecular Ecology*, **12**, 2003–2009.
- Dallas JF, Coxon KE, Sykes T *et al.* (2003) Similar estimates of population genetic composition and sex ratio derived from carcasses and faeces of Eurasian otter *Lutra lutra*. *Molecular Ecology*, **12**, 275–282.

- Ernest HB, Penedo MCT, May BP, Syvanen M, Boyce WM (2000) Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Molecular Ecology*, **9**, 433–441.
- Ernest HB, Rubin ES, Boyce WM (2002) Fecal DNA analysis and risk assessment of mountain lion predation of bighorn sheep. *Journal of Wildlife Management*, **66**, 75–85.
- Gerloff U, Schlotterer C, Rassmann K *et al.* (1995) Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living Bonobos (*Pan paniscus*). *Molecular Ecology*, **4**, 515–518.
- Goodman SJ (1998) Patterns of extensive genetic differentiation and variation among European harbor seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorphisms. *Molecular Biology and Evolution*, **15**, 104–118.
- Goossens B, Latour S, Vidal C *et al.* (2000) Twenty new microsatellite loci for use with hair and faecal samples in the chimpanzee (*Pan troglodytes troglodytes*). *Folia Primatologica* **71**, 177–180.
- Hedmark E, Flagstad O, Segerstrom P *et al.* (2004) DNA-based individual and sex identification from wolverine (*Gulo gulo*) faeces and urine. *Conservation Genetics*, **5**, 405–410.
- Høss M, Kohn M, Pääbo S, Knauer F, Schröder W (1992) Excrement analysis by PCR. *Nature*, **359**, 199.315.
- Kelly BP (1996) Live-capture of ringed seals in ice-covered waters. *Journal of Wildlife Management*, **60**, 678–684.
- Kelly BP, Quakenbush LT (1987) Trained dogs and wild seals. Whalewatcher, *Journal of the American Cetacean Society*, **21**, 8–11.
- Lucchini V, Fabbri E, Marucco F *et al.* (2002) Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. *Molecular Ecology*, **11**, 857–868.343.

- Manchester KL (1995) Calculation of A260/280 ratios for measurement of purity of nucleic acids. *BioTechniques*, **19**, 208–210.
- Maudet C, Luikart G, Dubray D, Von Hardenberg A, Taberlet P (2004) Low genotyping error rates in wild ungulate faeces sampled in winter. *Molecular Ecology Notes*, **4**, 772–775.
- Miller CR, Joyce P, Waits LP (2002) Assessing allelic dropout and genotype reliability using maximum likelihood. *Genetics*, **160**, 357–366.348.
- Miller LM, Kapuscinski AR (1997) Historical analysis of genetic variation reveals low effective population size in a northern pike (*Esox lucius*) population. *Genetics*, **147**, 1249–1258.
- Morin PA, Chambers KE, Boesch C, Vigilant L (2001) Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). *Molecular Ecology*, **10**, 1835–1844.
- Pääbo S (1990) Amplifying ancient DNA. In: *PCR Protocols* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ), pp. 159–166. Academic Press, San Diego, CA.
- Palomares F, Godoy JA, Piriz A, O'Brien SJ, Johnson WE (2002) Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and 357 feasibility for the Iberian lynx. *Molecular Ecology*, **11**, 2171–2182.
- Parsons KM (2001) Reliable microsatellite genotyping of dolphin DNA from faeces. *Molecular Ecology Notes*, **1**, 341–344.
- Parsons KM, Dallas JF, Claridge DE *et al.* (1999) Amplifying dolphin mitochondrial DNA from faecal plumes. *Molecular Ecology*, **8**, 1766–1768.
- Perry EA, Carr SM, Bartlett SE, Davidson WS (1995) A phylogenetic perspective on the evolution of reproductive behavior in pedophilic seals of the Northwest Atlantic as indicated by mitochondrial DNA sequences. *Journal of Mammalogy*, **76**, 22–31.
- QIAGEN (2001) *DNeasy Stool Kit Handbook*. QIAGEN. Valencia, CA.

- Reed JZ, Tollit DJ, Thompson PM, Amos W (1997) Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Molecular Ecology*, **6**, 225–234.
- Rudnick JA, Katzner TE, Bragin EA, Rhodes OE, DeWoody JA (2005) Using naturally shed feathers for individual identification, genetic parentage analyses, and population monitoring in an endangered Eastern imperial eagle (*Aquila heliaca*) population from Kazakhstan. *Molecular Ecology*, **14**, 2959–2967.
- Säisä M, Koljonen ML, Tahtinen J (2003) Genetic changes in Atlantic salmon stocks since historical times and the effective population size of a long-term captive breeding programme. *Conservation Genetics*, **4**, 613–627.
- Scandura M (2005) Individual sexing and genotyping from blood spots on the snow: a reliable source of DNA for non-invasive genetic surveys. *Conservation Genetics*, **6**, 871–874.
- Smith TG, Stirling I (1975) The breeding habitat of the ringed seal (*Phoca hispida*). The birth lair and associated structures. *Canadian Journal of Zoology*, **53**, 1297–1305.
- Swanson BJ, Rusz PJ (2006) Detection and classification of cougars in Michigan using low copy DNA sources. *American Midland Naturalist*, **155**, 206–217.
- Taberlet P, Griffin S, Goossens B *et al.* (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acid Research*, **24**, 3189–3194.
- Taberlet P, Camarra JJ, Griffin S *et al.* (1997) Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Molecular Ecology*, **6**, 869–876.

Conclusions

Three types of telemetry – VHF radio tracking, under-water acoustic tracking, and satellite tracking – allowed us to develop the first comprehensive picture of annual movements and habitat use by ringed seals. We pioneered the attachment of small satellite-linked transmitters to the hindflippers of seals and, as a result, were able to track some ringed seals between successive breeding seasons. Tag loss was higher than expected (50%), apparently do to the failure of the adhesive attaching the transmitters to the plastic tags.

The tracking records indicated that ringed seals are confined to small home ranges – most less than 3 km² – from freeze up until break up. In some portions of the species' range that period can last 9 months. After break up, the seals typically range 200 km or more from their winter sites, and some seals range over 1000 km from those sites. Between freeze up the following fall and January, the seals returned to the same breeding sites used the previous year. We located 12 individual ringed seals in successive breeding seasons, and they all returned to the same sites used previously. No seals that we tracked showed evidence of breeding at a different site in successive years. Thus, while they range widely when foraging in summer and fall, ringed seals show strong fidelity to their ice-bound breeding sites.

Fidelity to small home ranges, coupled with the ringed seal's small size, requires them to choose sites with sufficient prey densities to sustain them and their young for more than half of the year. More detailed studies of the ringed seals' prey and foraging behavior in the subnivean period are needed. The costs to ringed seals of being displaced during the subnivean period by human activities (Frost et al. 1988; Kelly et al. 1988; Moulton et al. 2005) and/or environmental change (Kelly 2001) also should be re-evaluated in light of their fidelity to these small, under-ice home ranges. We and others assumed in previous analyses that displacement from those sites was non lethal, and that conclusion may prove untenable.

Fidelity to breeding sites raised the possibility that ringed seals may be subdivided into many demographically distinct subpopulations (Dizon et al. 1992). If seals return to their own natal sites to breed, gene flow between groups would be limited, the potential for local depletion or extinction correspondingly higher. We were able to follow movements of individuals for as long as 14 months, a sufficient period to establish inter annual fidelity to breeding sites but insufficient to determine whether seals return to their own natal sites.

Natal philopatry and rates of gene flow can be inferred from genetic diversity, and molecular markers from a large sample of ringed seals were analyzed by Davis (2004). He reported no evidence of stock structuring among ringed seals, but his samples from the subsistence harvest were from seals taken mostly outside of the breeding season. Because seals move extensively outside of the breeding season, the harvest locations do not reflect breeding locations. Therefore, we sought a method of sampling DNA from ringed seal in their breeding locations.

Ringed seals undergo an annual catastrophic molt on the ice shortly after breeding. Large quantities of shed skin is left on the ice next to breathing holes in the same area occupied throughout the subnivean period including the breeding season. We developed protocols for collecting and preserving that skin and for extracting DNA from it (Swanson et al. 2006). In NPRB project 631, we are applying those methods to collecting and analyzing samples of ringed seal DNA collected from around the Arctic. We expect to estimate rates of gene flow and determine the scale at which ringed seal populations are structured. Those results will be valuable to the National Marine Fisheries Service and the Ice Seal Committee as they consider management strategies for ringed seals.

The results also will be useful in assessing threats to ringed seal populations. We predict that diminishing sea ice will result in introgressive hybridization between ice-associated marine mammals at several taxonomic levels (Kelly et al. in review). Hybridization is likely between currently distinct populations of ringed seals and, possibly, between ringed seals and congeners, most likely spotted seals (*Phoca largha*) and harbor seals (*P. vitulina*).

Publications

Swanson, B. J., B. P. Kelly, C. K. Maddox, and J. R. Moran. 2006. Shed skin as a source of DNA for genotyping seals. *Molecular Ecology Notes* 6:1006-1009.

Kelly, B. P., A. R. Whiteley, and David A. Tallmon. *in review*. Diminished sea ice cover and the potential for extinctions by introgressive hybridization. *Evolutionary Applications* (submitted June 2008).

Kelly, B. P., O. H. Badajos, M. Kunasranta, J. R. Moran, M. Ponce, D. Wartzok, and P. Boveng. in prep. Seasonal home ranges and fidelity of breeding sites among ringed seals. To be submitted to *Ecological Monographs*.

Outreach

Web page developed:

Peard Bay 2005 – Alaska Ringed Seal Project <http://www.nanuuq.info/peardbay.html>

Arctic Seals Vulnerable to Fast Pace of Change

<http://www.earthsky.org/radioshows/52291/fast-pace-of-arctic-change-affecting-seals>

Conference presentations:

International Committee on Arctic Research Planning II, Copenhagen - 11 November 2005

Alaska Marine Science in Alaska - 25 January 2006

Strengthening Cities: Mayors Respond to Global Climate Change, Girdwood – 16-18 Sep. 2006

Wildlife Society, 13th Annual Conference, Anchorage, Alaska – 23 – 27 September 2006

Carnivores 2006: Habitats, Challenges and Opportunities, St. Petersburg, FL. – 12-15 Nov 2006

Marine Mammals of the Holarctic, St. Petersburg, Russia - September 2006

Alaska Marine Science Symposium, Anchorage, Alaska - January 2007

Alaska Chapter of the Wildlife Society Annual Conference, Juneau, AK - April 2007

Society of Conservation Biology, Port Elizabeth, South Africa – 1-7 July 2007

AAAS – Arctic Division meeting, Anchorage, Alaska – 23–26 September 2007

CMU Chapter of The Wildlife Society, Central Michigan University, MI - 10 October 2007

Animal-borne Imaging Symposium, Washington, DC – 11 October 2007

Seals & Society; international symposium, Vaasa, Finland – 16–18 October 2007

National Science Teachers Association, Birmingham, Alabama - 7-8 December 2007

American Geophysical Union meeting, San Francisco, California - 10-14 December 2007

Community Meetings:

Alaska Native Ice Seal Committee, Fairbanks - 20 July 2005

Ice Seal Committee, First Alaskan's Institute, Anchorage - 31 January 2006

North Slope Borough Fish and Game Committee, Barrow - 29 March 2006

Northwest Alaska Climate Change Workshop, Kotzebue - 24-25 May 2006

Ice Seal Committee meeting, Anchorage, Alaska – 24 October 2006

North Slope Borough Fish and Game Committee, Barrow – 20 December 2006

Presentation to the IRA, Unalakleet, Alaska – 24 April 2007

North Slope Borough Fish and Game Committee, Barrow - 4 December 2007

Presentations at Festivals/Events:

- Juneau Rotary Club, Juneau - 6 September 2005
- Marine Mammal Commission meeting, Anchorage - 13 October 2005
- U. S. Arctic Research Commission, Portland - 28 October 2005
- University of Alaska Anchorage Bookstore - 25 January 2006
- Office of Polar Program, National Science Foundation - 7 March 2006
- Climate Change Seminar, University of Alaska Southeast - 10 March 2006
- Pacific Rim Conference - 30 March 2006
- Earth Day, Mendenhall Visitor's Center, Juneau - 22 April 2006
- Glacier Bay National Park and Preserve - 24 April 2006
- Auke Bay Laboratory, NOAA, Special Seminar - 22 June 2006
- Climate Camp: Alaska - 30 October – 1 November 2006
- Municipal League of Alaska (keynote address), Juneau, Alaska – 15 November 2006
- Testimony before U.S. Senate Subcommittee on *Private Sector and Consumer Solutions to Global Warming and Wildlife Protection*, Washington, DC – February 2007
http://epw.senate.gov/public/index.cfm?FuseAction=Hearings.Testimony&Hearing_ID=7efcd166-802a-23ad-4634-25057d9d08bf&Witness_ID=9ee2e8ca-5581-47c3-a857-66e172ee22e4
- Panel Discussion on Global Warming and Wildlife, (Gerald Kooyman, Brendan Kelly, Greg Marshall), National Geographic Society, Washington, DC – 13 October 2007

Workshop Participations:

- Climate Change workshop, University of Washington, Seattle - 28-29 July 2005
- Arctic Ocean Synthesis meeting - Honolulu - 16-17 February 2006
- Shelf Basin Interactions PI's meeting - Kauai, Hawaii - 25-28 February 2006
- NOAA EPP Workshop, Florida A&M University, Tallahassee, Florida – 30 Oct - 1 Nov 2006
- Collaborations on Climate Change Research, Univ. Alaska Workshop – 10 November 2006
- Workshop on Monitoring Arctic Marine Mammals, Valencia, Spain – 4 - 6 March 2007
http://sitios.cac.es/microsites/belugas_workshop/docs/Arctic.pdf
- SAP4.3 Authors' meeting (contributed *Sea ice ecosystem*), Boulder - 14-15 November 2007

Presentations in Schools (K-12, undergraduate):

- Science for Alaska Lecture Series, University of Alaska Anchorage - 23 January 2006
- Science for Alaska Lecture Series, Fairbanks, Alaska - 14 February 2006

Research Experiences for Undergraduates seminar, Univ. Alaska Southeast – 9 August 2006

Mentoring high school student, Emily Johnson in Science Fair project – 21 September 2006

Classroom presentation at Long Branch Elementary, Arlington, Virginia – 12 April 2007

Adolescent Montessori Program, Juneau, Alaska – 28 September 2007

Press Articles (Newspaper/Journal/Newsletter):

Associated Press interview - 12 April 2006

Associated Press interview - 3 Jan 2007

<http://www.adn.com/news/alaska/story/8671415p-8565319c.html>

Interview (Ocean Conservancy) for *Snowed in; ringed seals scratch out a life in the harshest of worlds*, – Spring 2007

“Rapid climate change and the sea ice ecosystem” in World Wildlife Fund’s Arctic Bulletin – April 2007. <http://assets.panda.org/downloads/ab0107.pdf>

Science News interview - 5 Oct 2007 (<http://www.sciencenews.org/articles/20071201/bob9.asp>)

Factsheets Produced:

Consultation to Minnesota Zoo, (consult on seals and polar bears for exhibit) – 8 November 2007

Video Produced:

Ice Masters, a documentary featuring this project, National Geographic Society, Washington, DC
(Public showing and discussion) – 9 October 2007

Radio/Television Interviews.

Video interview: Ron Meyer (documentary film on climate change) – 11 September 2006

Video interview: Flying Fast Productions (climate change documentary) – 11 October 2006

Interview: Public Broadcasting Service for documentary on climate change – 29 June 2007

Video interview: AdvanBridge Inc. (Japan) on sea ice and polar bears, Juneau – 17 Nov 2007

Radio interview: *Earth and Sky*, National Public Radio, Washington, DC – 20 December 2007

<http://www.earthsky.org/radioshows/52291/fast-pace-of-arctic-change-affecting-seals>

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Literature cited

- Dizon, A. E., C. Lockyer, W. F. Perrin, D. P. DeMaster, and J. Sisson. 1992. Rethinking the stock concept: a phylogeographic approach. *Conservation Biology* 6:24-36.
- Frost, K.J., and L.F. Lowry. 1988. Effects of industrial activities on ringed seals in Alaska, as indicated by aerial surveys, p. 15–25. In W.M. Sackinger, M.O. Jeffries, J.L. Imm and S.D. Treacy [eds.], *Port and Ocean Engineering Under Arctic Conditions, Vol. II: Symposium on Noise and Marine Mammals*. Geophysical Institute, University of Alaska Fairbanks.
- Kelly, B. P., A. R. Whiteley, and David A. Tallmon. *in review*. Loss of sea ice and the potential for extinctions by introgressive hybridization. *Science* (submitted April 2008).
- Kelly, B. P., J. J. Burns, and L. T. Quakenbush. 1988. Responses of ringed seals (*Phoca hispida*) to noise disturbance. Pages 27-38 *in* W. M. Sackinger, M. O. Jeffries, J. L. Imm, and S. D. Treacy, eds. *Port and Ocean Engineering under Arctic Conditions. Vol. II. Symposium on noise and marine mammals in ice-covered waters*. Geophysical Inst., Univ. Alaska, Fairbanks.
- Moulton, V.D., W.J. Richardson, R.E. Elliott, T.L. McDonald, C. Nations and M.T. Williams. 2005. Effects of an offshore oil development on local abundance and distribution of ringed seals (*Phoca hispida*) of the Alaskan Beaufort Sea. *Mar. Mammal Sci.* 21(2):217–242.
- Swanson, B. J., B. P. Kelly, C. K. Maddox, and J. R. Moran. 2006. Shed skin as a source of DNA for genotyping seals. *Molecular Ecology Notes* 6:1006-1009.