Biology of the Ribbon Seal in Alaska

Report to:

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INTRODUCTION

Four species of seals live in association with sea ice in Alaska; these are the ringed seal (*Phoca hispida*), spotted seal (*P. largha*), bearded seal (*Erignathus barbatus*), and ribbon seal (*Histriophoca fasciata* or *Phoca fasciata*). Ribbon seals, the focus of this paper, range from the Sea of Japan and the Sea of Okhotsk eastward into the Bering Sea and northward into the Chukchi Sea. In the Bering Sea, ribbon seals concentrate along the front of the ice pack in late winter and spring (Burns 1981). They rely on sea ice to provide a platform for pupping, nursing, and molting. Pupping occurs between early April and mid-May; nursing lasts three to four weeks. Breeding and molting occur before the sea ice recedes (Burns 1981). The distribution of ribbon seals shifts northward as sea ice recedes in May and June. When sea ice melts, the majority of the population probably becomes pelagic in the central Bering Sea, although some seals follow receding ice into the Chukchi Sea (Burns 1981).

The status of the ribbon seal is currently under scrutiny due to concerns regarding how changes in sea ice habitat may be affecting the species (50 CFR 223 and 224, March 28, 2008). Sea ice is changing in thickness, persistence, and distribution (Rigor and Wallace 2004, Comiso 2006, Serreze *et al.*2007). Evidence also indicates that oceanographic conditions have been changing in the Bering and Chukchi Seas (Niebauer 1980, 1983, 1988, Trenberth 1990, Ebbesmeyer *et al.*1991, Grebmeier *et al.*2006), which suggests changes in the ecosystem may be occurring as well. Of the ice seals, ribbon seals are the least common in Alaskan waters and because of this are the least understood. Population estimates for ice seals are not easily attainable due to their wide distribution and the problems related to marine mammal surveys in remote, ice-covered waters. However, there is some information regarding ribbon seal abundance. In 1961, the Russians began a commercial harvest of ribbon seals in the Bering Sea (Burns 1986). Before sealing began, the population was estimated to have between 100,000 to 120,000 ribbon seals (Fedoseev 2000). The commercial harvest is believed to have removed up to 13,000 ribbon seals annually until 1968, when the harvest was reduced to 6,200. The harvest was further reduced to 3,000 seals in 1969 (Burns 1981, 1986). The harvest is believed to have caused a population decline. Fedoseev (2000, citing Shustov 1969) estimated there were between 80,000–90,000 ribbon seals in the Bering Sea during the winter of 1963–1964. By the winter of 1969–1970, the population had decreased to approximately 60,000 ribbon seals (Burns 1981, citing Shustov 1972). Since then, the population is believed to have rebounded. Burns (1981) estimated the population had grown to between 90,000 and 100,000 by 1981. By 1987, Fedoseev (2000) estimated that the population had grown to approximately 140,000 and had completely recovered from commercial sealing. Currently, there are no estimates of abundance available for ribbon seals. The current harvest in Russia is also unknown.

The Alaska Department of Fish and Game (ADF&G) has been collecting information from the Alaska Native subsistence harvest on ribbon seals for over 40 years. We rely on the cooperation of several coastal communities to provide information and

samples of seal tissues for information on the health and status of seal populations. With these samples, we monitor species distribution, harvest availability, contaminant levels, and indices of population status (*e.g.*, pregnancy rates, age at first reproduction, growth rates, and body condition). Villages participating in the biomonitoring program span from Hooper Bay in the Bering Sea to Kaktovik in the Beaufort Sea (Fig. 1), an area that covers virtually the entire range of ribbon seals along the Alaska coast.

The purpose of this paper is to make unpublished data, collected by the State of Alaska, available for researchers, managers and others interested in ribbon seals. Here we summarize and assess ribbon seal samples collected between 1962 and 2008, a span of 46 years. The data that have been collected are diverse but important for gaining a larger understanding of ribbon seal ecology. These data evaluate growth rates, body condition, age distribution, productivity (age at first reproduction and pregnancy rate), diet composition, contaminants (trace metals, organochlorines, and new compounds), disease, genetics, and local knowledge. When possible, we focus on how conditions have changed over time (growth rates, body condition, age distribution, productivity, and diet). For data types that have small sample sizes or were recently added to the biomonitoring program (contaminants, disease, genetics, and local knowledge) we summarize current conditions for use as baselines in future studies.

METHODS

Sampling years

Ribbon seals were sampled between 1962 and 2008; however, sampling effort/opportunity was not consistent. The majority of ribbon seals sampled were harvested in 1967, 1978, and 2003 (Fig. 2). The largest single sampling year was 1967. The winter of 1966–67 was atypical and characterized by warmer temperatures, storms, and prevailing south winds. Sea ice was unusually fragmented and the ice edge was several hundred miles north of its usual location along the shelfbreak; this brought large numbers of ribbon seals north to St. Lawrence and Little Diomede islands (Burns 1968). Burns (1968) estimated that approximately 1,100 ribbon seals were harvested in 1967. In the years 1976, 1977, 1978, and 1979, the sample provided by the subsistence harvest was supplemented by biologists collecting seals during research cruises on the ship *M/V Surveyor*. Sixty-one ribbon seals were collected and data from some of these seals were used in our analyses. When examining trends in population attributes over time, we grouped samples into three distinct time periods, the 1960s, 1970s, and the 2000s.

Collection and handling

Biological information collected included location, date harvested, date sampled, species, sex, and measurements. Measurements included standard length as defined by the American Society of Mammalogists (1967), which is the straight line distance measured from nose to tip of tail with seals on their backs. Blubber thickness was measured through a small incision to the sternum midway between the front flippers and

axillary girth was measured with a soft tape placed under the foreflippers at the level of the axillae (McLaren 1958). Samples collected included one of the mandibles, the female reproductive tract, the whole stomach, and liver, kidney, blubber, and skin tissue. Samples were frozen in the field and shipped frozen to ADF&G in Fairbanks for processing.

Ageing— For specimens collected in the 1960s and 1970s, ages were determined using two methods: counting cementum layers of decalcified sections of canine teeth (Hewer 1960, Mansfield and Fisher 1960, Burns 1969) and counting the number of growth ridges on claws (McLaren 1958, Burns 1969).

Canine teeth were extracted from the jaw and stored in Loess' solution. Teeth were decalcified using a solution of 3% HCL. Teeth were decalcified until they were "rubbery" and then rinsed in tap water for 3–4 hrs (Burns 1969). After rinsing, teeth were cut in cross-section with a razor blade approximately two thirds of the way up the root. The root end was clamped in a mirotome and frozen with a commercial fluorinated hydrocarbon (Cyrokuik). Sections approximately 40 microns in thickness were cut and stained for 10 min using alum hematoxylin, purchased from Paragon C. & C. Co. in New York. Sections were then rinsed in tap water; excess stain was removed by placing sections in dilute sulfuric acid (7 drops acid:250 ml water) until they appeared light red. Sections were then transferred to a dilute solution of lithium carbonate until blue and then rinsed in water (Burns 1969). Stained sections were placed on a slide, blotted dry and dried overnight. A few drops of xylene solution and a cover slip were added. Cementum layers, deposited annually, were then counted to determine age (Hewer 1960, Mansfield and Fisher 1960, Stewart *et al.*1996).

Two claws were obtained from each seal and treated as independent specimens (Burns 1969). Claws were soaked in a solution of 50% isopropyl alcohol and zylol for at least two days prior to aging. Age was determined while the claws were wet. Soaking increased contrast and made the ridges more distinct. The formation of ridges is thought to be produced by physiological stress, which occurs for ice seals during spring and early summer when birth, lactation, breeding, decreased feeding, and molting occur. Age was determined from claws by counting each ridge but excluding the ridge that occurs at birth (Burns 1969).

For specimens collected since 2000, ages were determined by canine teeth only using the following method. Mandibles were soaked in hot water for at least 1 hr before extracting a canine tooth using a tooth extractor. Canines were cut into sections, 14 microns thick, and stained with Giemsa histological stain by Matson's Lab, Milltown, MT. The growth layer groups of cementum were counted for age determination according to Stewart *et al.*(1996) either by L. Dehn or by Matson's Lab.

Morphometrics

Growth rate— We investigated the change in ribbon seal growth rates over time by fitting von Bertalanffy growth curves (*e.g.*, Schnute 1981, McLaren 1993) to age-at-length data. The model is:

$$L_x = L_{inf}\left(1 - e^{-a(x-x_0)}\right)^b,$$

Where

L_x is the standard length of harvested individuals,
L_{inf} length at infinite age (*i.e.*, asymptotic length),
a and b are rate parameters that define the rate at which growth approaches asymptotic length,
x is the empirical age of individuals, determined by teeth and/or claws, and
x_0 is an adjustment for where the curve crosses the x-axis. Because of prenatal growth, individuals are not length 0 at birth.

L_x and x are vectors of empirical data, from harvested seals; L_{inf}, a, and b are estimated parameters. McLaren (1993) recommends setting x_0 constant, rather than estimating x_0 from the Bertalanffy growth curve. Using real biological information to calculate x_0 is more accurate than estimating x_0 and this helps anchor the rest of the curve. We follow McLaren's (1993) recommendation and use -0.63 years for x_0. Ribbon seals have a gestation period of 11 months, with a delayed implantation of 3.5 months (Burns 1986); hence, the implied time of implantation is 0.63 years (*i.e.*, 11 - 3.5 = 7.5 months or 0.63 years).

We chose to use data for seals less than one year of age. McLaren (1993) chose not to include seals within their first year of life, because the growth is linear during this time for many pinnipeds and is therefore not well fit by growth curves. We agree that this would be an issue if seals were measured across a wide range of time periods; however, 74% of our seals less than one year of age were sampled within the same month (May) and 99% were sampled within April, May, or June. In addition, standard length was not correlated with month of sampling (correlation coefficient = 0.08). Therefore, we did not remove individuals less than a year old.

We used the raw data collected by ADF&G since 1963, some of which has been published (Burns 1981). Ages were determined by counting annual ridges on seal claws and by counting cementum layers in sectioned canine teeth. In the 1960s, some ribbon seals (48 of 137) were only aged by counting annual ridges in the claws. With the exception of two outliers, ages determined from claws overlapped ages determined by teeth. To ensure that including claw ages within our sample was not biasing our results, we repeated our analysis using only tooth ages.

We estimated L_{inf}, a, and b within a Bayesian framework using Gibbs sampling (Congdon 2003, Gelman *et al.*2004) in WinBugs (Speigelhalter *et al.*2003). Bayesian methods use simulations to describe the probability distribution of a parameter, such as

L_{inf}, given the data (Gelman *et al.*2004). Each simulation is commonly referred to as a 'chain'. We ran four chains, 50,000 iterations each, to confirm that all chains converged on the same solution. We discarded the first 10,000 iterations (*i.e.*, the 'burn-in') to remove the effect of initial values on the posterior distribution. To confirm that our model was converging on a single, stable solution, we examined Gelman-Rubin plots (Gelman and Rubin 1992) and the iterative histories for each parameter in each chain.

We used standard lengths and ages from seals collected in the 1960s, 1970s, and 2000s. We were primarily interested in determining if ribbon seal growth has changed between time periods. However, relatively few old seals (>15 years of age) were collected in the 1960s and 1970s, and we were forced to estimate L_{inf} for the data pooled across all time periods. In effect, our analysis assumes that all seals are capable of reaching the same asymptotic length, regardless of when they were born.

After estimating L_{inf} for the pooled data, we then treated L_{inf} as a constant and estimated a and b, when comparing models of ribbon seal growth. Rather than directly comparing estimates of a and b, which are difficult to decipher without plotting, we treated different models of seal growth as hypotheses. Models were selected using Deviance Information Criterion (DIC; Spiegelhalter *et al.*2002). DIC is equal to the -2 log likelihood of the model, calculated with the posterior means of the model parameters, plus 2 times the effective number of parameters. DIC are used for model selection in a similar fashion as Akaike Information Criterion (Burnham and Anderson 2002); models greater than 4 DIC units from the best approximating model are considered to have little to no statistical support.

We compared 7 models (Table 1); five models combined time periods in different ways. For example, one model assumed growth rates were the same during all time periods and another assumed growth rates differed in all time periods. Two additional models examined gender specific growth rates. One assumed that growth varied only by gender and not time period. One included growth rates that varied by time period and gender, but for the 1960s and 1970s only, as there was not enough data to estimate gender effects for data collected since 2000.

Body condition—To assess body condition, we chose to use a volumetric index described by Parsons (1977) and Gales and Renouf (1994). Many indices of body condition are available; however, most simple indices are not well correlated with seal blubber content. For example, Ryg *et al.*(1990*a*) compared condition indices to true blubber content in a sample of 132 ringed seals and found that the two most common indices of body fat, sternal blubber thickness and the Smirnov index (*i.e.*, max girth/standard length * 100; Smirnov 1924, Sergant 1973) were not strongly correlated to blubber content (sternal blubber thickness: R^2=0.46; Smirnov index: R^2=0.39). One method described by Gales and Renouf (1994) assumed that seals were essentially cylinders; the volume of an inner cylinder, which excludes blubber thickness, is subtracted from an outer cylinder, which includes blubber thickness. The height of the cylinder is equal to seal length, the radius of the outer cylinder is calculated from seal girth, and the radius of the inner cylinder is calculated as the radius of the outer cylinder

minus blubber thickness. For harp seals (*Phoca groenlandica*), Gales and Renouf (1994) found this index more strongly correlated with blubber content (% of body mass; R^2=0.948 for max girth and sternum blubber thickness; R^2=0.892 for axillary girth and sternum blubber thickness) than sternal blubber thickness alone (R^2=0.471) or the Smirnov index (R^2=0.159). We used sternal blubber thickness, axillary girth, and standard length to calculate the inner and outer cylinders for ribbon seals.

Although better indices of body condition are available, they generally rely on measuring seal mass (*e.g.*, Ryg *et al.*1990*a*, Arnould 1995); however, mass was generally not measured in our sample. Other indices depend upon the volume of multiple cylinders (*e.g.*, Gales and Renouf 1994) and require multiple measurements of girth. Hence, the volumetric index is expected to be the best index available, given the data at hand.

Due to sample size limitations and skewed distributions, we used a non-parametric test for comparing sample means. We rank transformed body condition indices and then used ANOVA to test for differences by gender (male vs. female) and time period (1960s, 1970s, and 2000s). Because seals are expected to gain and lose mass seasonally (Ryg *et al.*1990*b*), it is important to control for season when comparing separate time periods. We limited our comparisons to seals sampled in the month of June, as samples collected in other months were not well represented within all time periods.

Age Distribution

We characterized the age distribution of seals harvested in the 1960s, 1970s, and 2000s by plotting the cumulative proportions of ribbon seals in each age class. We acknowledge that we must make inferences cautiously from harvest data, as hunter preference may affect how the age distribution of the harvest is biased relative to the true age distribution of the population. However, because hunters cannot distinguish the age of mature seals by sight and because hunters do not forego opportunities to harvest ribbon seals, the sample of adult seals should be effectively random. Changes in which seals are available for harvest may also result in bias. However, changes in population growth or structure are expected to manifest in the harvest.

To compare age distributions over time and to identify where age distributions deviated, we categorized our sample into five separate groupings and calculated chi-square statistics. Category thresholds were < 5, < 10, >10, > 15, and > 20 years of age and were not mutually exclusive. For example, the first categorization compared the proportion of seals that were < 5 years of age to all seals $\geq$ 5; the second categorization compared seals that were < 10 years old to all seals $\geq$10. We did not include pups in our analysis, as all but two pups were harvested in 1967. In 1967, we believe that a warm winter and south winds relocated large ice pans from the Russian coast to the within hunting range of St. Lawrence Island, Diomede Island, and the Alaska coast. These ice pans were occupied by many ribbon seals, including mother/pup pairs, and many pups were harvested (Burns 1968). These pups are not typically available to hunters and a

large harvest of pups was not observed in other years. Hence, by removing pups from the analysis of age distributions, we removed a large source of availability bias.

Age at Sexual Maturity

We evaluated the age at sexual maturity and pregnancy rates for samples collected since 2000. Reproductive tracts were evaluated for status (nulliparous, primiparous, or multiparous)[1] and condition (*e.g.*, pregnant, not pregnant) by sectioning ovaries, identifying *corpora lutea* and *corpora albicantia*, and examining the condition of uterine horns (McLaren 1958, Johnson *et al.*1966, Smith 1973). We defined age at sexual maturity as the age at which the first ovulation occurred (McLaren 1958, Tikhomirov 1966, Smith 1973). Due to the delay between conception and implantation in pinnipeds (Harrison and Kooyman 1968) there are several months where pregnancy cannot be determined by the presence of a fetus. The presence of a *corpora lutea* indicates that the female ovulated but pregnancy can not be confirmed during this time period. We considered all females with a *corpora lutea* that were harvested from May to September to be pregnant. If each ovulation does not result in a pregnancy the pregnancy rate will be inflated.

To quantify the average age of sexual maturity we calculated the average age of primiparous females in our sample. This statistic will be biased high, because it does not account for females of similar ages that have not ovulated. Better approaches for estimating the average age of ovulation require an adequate sample size within intermediate age classes (*i.e.*, age classes that have a mix of mature and immature females). For example, logistic regression or the equations provided by DeMaster (1978) are expected to provide unbiased estimates of the average age of ovulation. However, we did not have enough data in the intermediate age classes for logistic regressions to converge on a solution or for DeMaster's (1978) approach to yield a useful estimate. Hence, we simply provide the average age of primiparous females as an index of the average age of ovulation and present the range of ages for nulliparous, primiparous, and multiparous females. Data collected in 1967 are summarized in Burns (1969); however, Burns defined sexual maturity as the age of initial pregnancy, which in some cases occurs later than the first ovulation. In order to compare time periods we re-analyzed Burns' (1969) data, using the age of first ovulation to define sexual maturity. We also present previously unpublished data collected between 1976 and 1979 by ADF&G.

Stomach Content analysis

Prey items collected from all but one of our specimens were published by Dehn *et al.* (2007) using their methods. One stomach with prey items was collected since that publication and is presented here. The stomach was thawed in the laboratory and the contents were sorted and weighed to the nearest 0.1 g. Contents were rinsed with freshwater through two sieves with mesh sizes of 1.0 mm and 0.5 mm and prey items were indentified to the lowest taxonomic level and weighed. Otoliths were identified to

[1] Nulliparous females are reproductively immature, primiparous females have ovulated only once, and multiparous females have ovulated more than once and given birth at least once.

the lowest taxonomic level by W. Walker at the National Marine Mammal Laboratory. Samples collected prior to 2003 are summarized in Frost and Lowry (1980).

Contaminants

Tissue preparation—For ribbon seals collected since 2000, we have sampled liver and blubber tissue for contaminants analysis. Liver and blubber tissue were clean-sampled at ADF&G following protocol established by the National Institute of Standards and Technology (Becker *et al.*1991) and contaminants were quantified by TDI – Brooks International, Inc., B&B Laboratories, Inc., College Station, TX. Individual seals were used for contaminants analyses only if liver, kidney, and blubber tissue were available in quantities that would allow the required sample amount for testing after clean sampling each tissue. A tooth was also required so that age could be related to results.

Trace metals analysis—Only liver tissue was analyzed for trace metals. Liver samples were homogenized with a meat grinder. An aliquot of approximately 100 g was weighed and freeze-dried and then further homogenized using a blender prior to extraction. Percent moisture was calculated by comparing the weight of the wet sample with the weight of the dry samples before a 0.5 g sample was extracted and digested in a microwave wet ash procedure using, H_2O_2, and HCl. Microwave digestion was used for all metals except As and Se.

Samples analyzed for As and Se were digested using magnesium dry ash digestion methods. This method uses methanol, HNO_3, HCl, and heat for digestion. After digestion As and Se were analyzed using Hydride Generation AA. Calibration was done at 0, 1.0, 5.0, 15.0 ppb and the QC check was 10.0 and a known Reference Sample. The 5.00 ppb standard was checked every 10^{th} sample and if the value differed by > 5% from 5.00 the instrument was recalibrated. If the value was > 10% different from 5.00 the last 10 samples were re-analyzed. Pb was analyzed using Graphite Furnace AA. Calibration was done at 0 and 1.0 ppb and then 3–5 standards were run to check the calibration. All other metals were analyzed using ICP on a Perkin-Elmer 4300 DV.

For total mercury, a 10 ml aliquot was removed immediately after dilution, HCl was added and concentrations were determined using Cold Vapor AA. Calibration was done at 0, 1.0, 5.0, 30.0 ppb and the QC checks were 10.0, 20.0, and a known reference sample. The 5.00 ppb standard was checked every 10^{th} sample and if the value differed by > 5% from 5.00 the instrument was recalibrated. If the value was > 10% different from 5.00 the last 10 samples were rerun.

OC analysis—Liver and blubber tissue were analyzed for organochlorines (*e.g.*, PCBs and pesticides). Tissue samples were homogenized using a stainless steel blender with titanium blades. Aliquots of approximately 15 g of wet tissue were chemically dried using Hydromatix® and extracted with 100% dichloromethane using a Dionex Accelerated Solvent Extractor (ASE200) operated at 100°C and 2,000 psi. The extracts are reduced to 3 mL by evaporative solvent reduction. A 100 µL aliquot is removed and weighed to determine lipid weight. The remaining sample portion is purified using

alumina/silica gel column chromatography and gel permeation column (GPC)/high performance liquid chromatography (HPLC). After HPLC purification, the eluents were reduced to 0.5 mL and analyzed for PCBs and pesticides by either gas chromatography/mass spectrometry (GC/MS) or gas chromatography/electron capture detector (GC/ECD).

A GC/ECD, coupled to two capillary columns, was used to resolve and detect chlorinated hydrocarbons (polychlorinated biphenyls and pesticides) in tissues. Samples were injected into a temperature-programmed GC/ECD, operated in splitless mode. The capillary columns are DB-5 (30 m x 0.25 mm ID and 25 μm film thickness) and DB-17HT (30 m x 0.25 mm ID and 0.15 μm film thickness). The DB-17HT column is used for analyte confirmation. A data acquisition system continuously acquired and stored all data for quantitation. This method is capable of producing data at parts-per billion and parts-per trillion concentrations. The surrogate spiking solution includes 4,4'-dibromooctaflurobiphenyl (DBOFB), 2,2',4,5',6 pentachlorobiphenyl (PCB 103), and 2,2',3,3',4,5,5'6 octachlorobiphenyl (PCB 198). Surrogate solution (100 μL) is added to all samples and quality control samples prior to extraction. Surrogate compounds are resolved from, but elute in close proximity to, the analytes of interest. The recovery of PCB 103 is used to correct analyte concentrations. Spikes, duplicates, and blanks were analyzed for quality control with each batch of 20 samples or less.

Disease

We tested serum from the blood of ribbon seals collected near Little Diomede Island for four diseases known to affect phocids; *Brucella abortus*, phocine herpesvirus-1 (PhHV-1), phocine herpesvirus-2 (PhHV-2), and phocine distemper virus (PDV). *Brucella* is known to cause reproductive problems in marine mammals, including placental infections and abortion (*e.g.*, Miller *et al.*1999). Zarnke *et al.* (2006) identified *Brucella* in harbor seals, in Alaska. PhHV-1 usually affects pups and immunocompromised or diseased adults (Zarnke *et al.*1997). In contrast to PhHV-1, PhHV-2 is not known to cause disease in phocids, however its antibodies have been detected in all the phocids within Alaskan waters (Zarnke *et al.*1997, Zarnke *et al.*2006). PDV is a morbillivirus known to cause large die-offs. PDV infected seals exhibit symptoms of respiratory distress and the most common post-mortem finding is pneumonia (Kennedy *et al.*1989). In Alaska, PDV has previously been identified in harbor seals (Zarnke *et al.*1997).

Blood collected from harvested seals was allowed to clot before being centrifuged and serum was transferred to sterile cryovials. The cryovials were stored at –20°C for several weeks and then at –40° C for several months before shipping to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) in Stillwater, OK for testing. For *Brucella* serum was screened for antibodies by using the standard card agglutination test (SCA). Samples that tested positive were retested using SCA, particle concentration fluorescence immunoassay, *Brucella* buffered antigen standard plate agglutination test, complement fixation test, standard plate test, and Rivanol test (MacMillan 1992). For PDV, PhHV-1, and -2, serum was tested for the presence of antibodies by using the

microplate virus neutralization test (Saliki and Lehenbauer 2001). Threshold titers of ≥ 8 were considered positive.

Genetics

Mitochondrial DNA, extracted from skin samples, were analyzed to determine genetic diversity and population structure by Greg O'Corry-Crowe at NMFS, Southwest Fisheries Science Center (for detailed methods see Kocher *et al.* 1989). A total of 600 base pairs (bp) of the mtDNA control region and adjacent proline tRNA gene were sequenced for ribbon seals (O'Corry-Crowe *et al.* 2003, O'Corry-Crowe and Bonin 2004).

Local Knowledge

Harvest data requires careful interpretation, as hunters do not randomly sample seals throughout their range. For example, changing preferences of hunters may confound the interpretation of seal distribution or age structure. Hunters may also have local knowledge that corroborates or aids the interpretation of data from the biomonitoring program. Hence, discussion with local hunters is critical for understanding how to interpret results of data analyses. We developed a questionnaire to collect information from villages participating in the bio-monitoring project. Questions were designed to determine the importance of the different seal species, whether changes had occurred in seal numbers, seal distribution, seal health, harvest methods, harvest timing, and local conditions. We used the responses to help us understand seal hunting practices and to identify topics that may need further investigation. The results help us understand whether changes observed in our sample collections are due to changes in seal numbers and behavior, or may be due to changes in harvest methods. Results obtained from the questionnaires were not intended to be definitive as they do not represent all of the hunters from each community; however majority responses give us a reasonable indication of hunter activity and preferences.

RESULTS

Importance in Subsistence Harvest

Ribbon seals were not consistently available for harvest by Alaskan villages. Between 2001 and 2008 we sampled 49 ribbon seals; 38 of these were from Little Diomede in 2003. Intermittent harvest records show that Gambell and Savoonga had similar harvests in 2005 with Gambell reporting 36 and Savoonga reporting 33 ribbon seals (Ahmasuk and Trigg 2007). In 1989, Shishmaref reported a harvest of 39 ribbon seals (Conger and Magdanz 1990). In most years, however the harvest of ribbon seals has been few or none. Answers to our local knowledge questionnaire indicated that ribbon seals were the rarest species in the harvests of all villages surveyed (Pt. Hope, Diomede, Shishmaref, Gambell, and Hooper Bay).

Morphometrics

Growth rate—Our analysis of growth rate by time period included 235 ribbon seals of known length and age (Fig. 3). Of these seals, 137 were harvested in the 1960s, 75 in the 1970s, and 23 since 2000. Of seals harvested in the 1960s, 48 were aged using claws. Asymptotic length (L_{inf}) was estimated from the pooled data (all time periods pooled) to be 155.7 cm (SD=2.4).

The only model supported by the data indicated that the growth rate of ribbon seals today was similar to the rate observed in the 1960s, but the growth rate in the 1970s was higher (Table 1, Fig 4a). The growth rate parameters are significantly different, as the 95% credibility intervals do not overlap (Table 2). The difference in standard length, between seals in the 1970s and other time periods rapidly increases between 1 and 3 years of age and then gradually decreases as seals approach a common asymptotic length. At age 3, seals were an average of 10.9 cm longer in the 1970s than the 1960s or 2000s (Fig. 4b).

The inclusion of seals aged by claws did not affect our analysis or results. Without the claw ages, asymptotic length (L_{inf}) was estimated from the pooled data to be 156.0 cm (SD=2.2). The only model supported by the data also indicated that growth rates of ribbon seals today are similar to rates observed in the 1960s, but were higher in the 1970s. However, the strength of evidence in support of this model was stronger, as the next best approximating model was 13.6 DIC units away. The maximum difference in standard length between time periods was also similar. Using only tooth ages, three year old ribbon seals were 10.1 cm longer in the 1970s than in the 1960s or 2000s.

Table 1. Models for ribbon seal growth rates in the 1960s, 1970s, and 2000s.

Model	**Effective parameters**	**DIC**	**Δ DIC**
1960s=2000s, 1970s	4.927	1855.74	0
All time periods differ	6.807	1859.79	4.05
1960s=1970s, 2000s	4.813	1861.82	6.08
1960s, 1970s=2000s	4.899	1864.69	8.95
Gender*period (60s and 70s only)	10.282	1865.95	10.21
Common growth rate	2.99	1869.16	13.42
Gender	4.921	1872.42	16.68

Table 2. Growth rate parameters from the best approximating model of ribbon seal growth.

Growth rate parameter	**Posterior means (95% credibility intervals)**	
	1970s	**1960s and 2000s**
a	-0.2174 (-0.2809, -0.1635)	-0.6289 (-0.8615, -0.4323)
b	0.2264 (0.1924, 0.2649)	0.6694 (0.3722, 0.8065)
L_{inf} **(constant)**	155.7	155.7

Body condition—We calculated the volumetric index of body condition for 24 seals collected in the 1960s, 23 collected in the 1970s, and 12 collected since 2000. The volumetric index was strongly correlated with sternal blubber thickness (correlation coefficient = 0.92). Blubber thickness ranged from 0.8 to 6.0 cm. Gender was not supported by the non-parametric ANOVA (Kruskal-Wallis *F* test: p=0.62) and was dropped from the model. Time period was supported as a significant source of variation in the data (Kruskal-Wallis *F* test: p <0.001). For blubber volume indices (cm^3/1000), ribbon seals averaged 29.23 (SE=1.86) units in the 1960s, 39.25 (SE=3.57) units in the 1970s, and 32.02 (SE=3.43) in seals collected since 2000. Statistical contrasts indicated that the body condition index was higher in the 1970s than the 1960s (p=0.03). Although mean body condition since 2000 was most similar to that in the 1960s (p=0.64) (Fig. 5), it is statistically indistinguishable from that in the 1970s (p=0.19).

Age Distribution

Not including pups, 220 seals were aged in the 1960s, 65 in the 1970s, and 44 in the 2000s. Age distributions were largely similar (Fig. 6). However, a larger proportion of older aged seals were harvested in the 2000s than the 1960s or 1970s (Fig. 7). The difference in the proportion of older aged seals was only statistically significant for individuals > 20 years of age (p<0.01), although the same pattern was present for seals > 15 years of age. There was also a higher proportion of seals < 5 years of age in the 1970s than the 1960s or 2000s (Fig. 7); however, this difference was not statistically significant (p=0.61). We repeated this analysis excluding claw ages from the 1960s. The results were virtually identical and the same patterns were supported statistically.

Productivity

Reproductive tracts were collected from 75 females collected between 1964 and 1968, 33 of which were sexually mature. Nulliparous females ranged in age from less than one year of age to three years. Primiparous females ranged from 1–5 years of age and multiparous females ranged from 3–23 years, although ages for two sexually mature females were unknown (Table 3). Of the 33 sexually mature females, 32 (97.0%) were

pregnant in the year they were harvested. The mean age of primiparous females was 2.3 years.

Table 3. Reproductive status of female ribbon seals harvested in Alaska during 1964–1967 by age class. These data are from Burns (1969); for comparison, we re-analyzed the data using first ovulation to determine sexual maturity.

	Nulliparous[1]		Primiparous[2]		Multiparous[3]		
Age	No.	%	No.	%	No.	%	Total in age class
Pup	35	100	0	0	0	0	35
1	3	60	2	40	0	0	5
2	3	50	3	50	0	0	6
3	1	25	1	25	2	50	4
4	0	0	0	0	6	100	6
5	0	0	1	50	1	50	2
6+	0	0	0	0	15	100	15
Total	**42**		**7**		**24**		**73**

[1] Nulliparous females are reproductively immature.
[2] Primiparous females have ovulated once.
[3] Multiparous females have ovulated more than once and given birth at least once.

Reproductive tracts were collected from 27 females collected on research cruises between 1976 and 1979, 21 of which were sexually mature. Nulliparous females ranged in age from one year of age to two years. There was one primiparous female that was 3-yrs-old and multiparous females ranged from 2–19 years. Most of the 2- and 3-yr-olds (67%) were multiparous as were all of the 4-yr-olds (Table 4). Of the 21 sexually mature females, 20 (95.2%) were pregnant in the year they were harvested. The average age of primiparous females was 3 years.

Reproductive tracts were collected from 20 females collected from the harvest between 2003 and 2007, 12 of which were sexually mature. Nulliparous females ranged in age from less than one year of age to 4 years. Primiparous females ranged from 2–4 years and multiparous females ranged from 3–13 years. Two of four 2-year-old females (50%) had ovulated (Table 5); one had ovulated but was not pregnant, and the other was pregnant. Of the 12 sexually mature females, 11 (92%) were pregnant in the year they were harvested. The average age of primiparous females was 3 years.

Table 4. Reproductive status of female ribbon seals collected from the *M/V Surveyor* in the Alaskan Bering Sea, 1976–1979.

	Nulliparous		Primiparous		Multiparous		
Age	No.	%	No.	%	No.	%	Total in age class
Pup	0	0	0	0	0	0	0
1	5	100	0	0	0	0	5
2	1	33	0	0	2	67	3
3	0	0	1	33	2	67	3
4	0	0	0	0	2	100	2
5+	0	0	0	0	14	100	14
Total	**6**		**1**		**20**		**27**

Table 5. Reproductive status of female ribbon seals harvested in Alaska during 2003–2007 by age class.

	Nulliparous		Primiparous		Multiparous		
Age	No.	%	No.	%	No.	%	Total in age class
Pup	1	100	0	0	0	0	1
1	3	100	0	0	0	0	3
2	2	50	2	50	0	0	4
3	0	0	1	50	1	50	2
4	1	20	2	40	2	40	5
5	0	0	0	0	1	100	1
6	0	0	0	0	2	100	2
13	0	0	0	0	1	100	1
Total	**7**		**5**		**7**		**19**

Stomach Content Analysis

A total of 46 stomachs were collected from ribbon seals harvested at Diomede, Point Hope, and Hooper Bay from 2002 to 2008. Of these 46 stomachs only 7 (15%) contained food. Prey identified included fish (pollock, *Theragra chalcogramma;* arctic cod, *Boreogadus saida*; and saffron cod, *Eleginus gracilis*) and shrimp (crangonid and pandalid species). Dehn *et al.* (2007) included the analysis of all but one of the seals in our database. The additional seal was collected at Point Hope in 2008 and contained otoliths from arctic and saffron cod.

Genetics

Variation in mitochondrial DNA (mtDNA) was examined using skin samples from 24 ribbon seals collected at Little Diomede Island (n = 22) and Hooper Bay (n = 2) in 2003. Samples represented eight males, 14 females, and two of unknown sex. Ages ranged from 0 to 25 with an average age of 5.1. A total of 600 base pairs (bp) of the mtDNA control region and adjacent proline tRNA gene were sequenced for ribbon seals.

Ribbon seals were found to posses very high levels of nucleotide and haplotype diversity with many individuals possessing unique haplotypes. Subdivisions within the population were not detectable.

Contaminants

Metals and other elements—Concentrations of 19 trace elements in liver tissue of nine ribbon seals were quantified (Table 6). Some of the elements are essential nutrients (Cu, Fe, Mg) and others are potentially toxic at high levels (Cd, Hg, Pb). Of metals that were potentially toxic, Hg was positively correlated with age (R^2=0.49) but geometric mean concentrations were similar for males (2.09 ng/g ww) and females (2.12 ng/g ww). Cd was not correlated with age (R^2=0.05), but females had a higher geometric mean concentration (9.25 ng/g ww) than males (1.13 ng/g ww). These results should be interpreted cautiously, as sample sizes are small. A 13 year old female, sampled near Point Hope in 2007, had the highest concentrations in 9 of 13 trace metals, including Cd, Hg, and Pb (Table 6).

Organochlorines—Organochlorines (OC) were quantified and summarized in the blubber (n=9) and liver (n=8) of ribbon seals sampled since 2000. We examined four compounds of hexachlorocyclohexane (HCH; Alpha-HCH, Beta-HCH, Delta-HCH, Gamma-HCH), seven compounds of chlordane (CHL; Heptachlor, Heptachlor-Epoxide, Oxychlordane, Alpha-Chlordane, Gamma-Chlordane, Trans-Nonachlor, Cis-Nonachlor), six compounds of dichlorodiphenyltrichloroethane (DDT;2,4'-DDD, 4,4'-DDD, 2.4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT), and 82 congener and congener groups of polychlorinated biphenyls (PCB) in both blubber (Appendix A) and liver tissues.

In order to make comparisons with other studies we present the levels of each congener analyzed in blubber tissue so that ΣPCBs can be calculated (Appendix). Of the 82 PCB congener and congener groups, three made up the more than half (51.8%) of the ΣPCBs in blubber. They were, in decreasing dominance 153/132 (22.4%), 101/90 (16.8%), and 138/160 (12.4%) (Appendix).

Table 6. Concentrations of trace metals in individual ribbon seals sampled since 2000. F/# = female/age in years. M/# = male/age. Coefficients of determination (R^2) are for females and males pooled. Geometric mean concentration and geometric standard deviation (SD) are for each gender. Highest levels for each metal are in bold italics. Metals that are potentially toxic are in bold.

Metals	Individual (gender/age)									Correlation w/age (R^2)	Geometric mean (SD)	
	F/2	F/3	F/4	F/6	F/13	M/2	M/5	M/9	M/21		Females	Males
Al	0.30	***7.22***	0.31	0.30	0.78	0.29	0.30	2.17	0.32	0.05	0.69 (3.96)	0.5 (2.67)
As	0.20	0.16	0.40	0.44	0.23	0.59	0.46	0.30	***0.96***	0.36	0.27 (1.55)	0.53 (1.62)
B	0.30	0.33	0.31	0.30	***0.63***	0.29	0.30	0.30	0.32	0.15	0.35 (1.38)	0.31 (1.04)
Ba	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.15	0.03 (1.04)	0.03 (1.04)
Be	0.01	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.15	0.02 (1.04)	0.02 (1.04)
Cd	9.69	12.66	11.59	3.14	***15.21***	0.79	2.25	2.20	0.42	0.05	9.25 (1.87)	1.13 (2.28)
Cr	0.07	0.08	0.19	0.07	0.08	0.07	0.76	0.08	0.08	0.02	0.09 (1.49)	0.14 (3.16)
Cu	4.77	11.25	11.35	5.92	***35.37***	7.64	6.69	5.13	13.06	0.21	10.5 (2.18)	7.65 (1.48)
Fe	933	1984	1324	2128	3819	770	1234	2199	420	0.01	1818.95 (1.7)	968.26 (2.02)
Hg	1.13	2.59	1.27	0.59	***18.06***	0.41	0.55	10.27	8.67	0.49	2.09 (3.72)	2.12 (5.65)
Mg	200	217.1	210.2	207.2	208.2	199	219.2	202.9	***222.1***	0.21	208.45 (1.03)	210.59 (1.06)
Mn	2.92	***6.23***	3.41	3.85	5.32	2.94	4.26	3.32	4.49	0.07	4.18 (1.37)	3.7 (1.22)
Mo	0.30	***0.98***	0.31	0.30	0.94	0.29	0.30	0.30	0.32	0.00	0.48 (1.89)	0.31 (1.04)
Ni	0.07	0.08	0.08	0.07	0.08	0.07	0.08	0.08	0.08	0.15	0.08 (1.04)	0.08 (1.04)
Pb	0.03	0.07	0.03	0.03	***0.13***	0.03	0.06	0.03	0.03	0.05	0.05 (1.91)	0.04 (1.41)
Se	3.28	3.61	1.74	2.46	***10.02***	1.47	1.79	6.95	5.78	0.45	3.47 (1.93)	3.21 (2.21)
Sr	0.09	0.03	0.03	0.09	0.09	0.09	0.06	0.12	***0.20***	0.72	0.06 (1.77)	0.11 (1.65)
V	0.07	0.33	0.08	0.30	***1.13***	0.24	0.15	0.66	0.26	0.19	0.23 (3.12)	0.28 (1.86)
Zn	43.5	57.4	56.7	48.5	***70.7***	42.3	46.5	54.1	49.4	0.09	54.63 (1.2)	47.89 (1.11)

Of the six compounds composing ΣDDT in blubber tissue, the most dominant compound detected was 4,4' DDE (88.0%). The geometric mean sum of DDTs (ΣDDT = Σ2,4'- and 4,4'-DDD and DDE and DDT) was 456.5 ng/g wet wt (geometric SD 2.06) and was similar in females (447.48 ng/g wet wt, geometric SD 2.02) and males (468.11 ng/g wet wt, geometric SD 2.34). In general, OC concentrations in liver tissue were at least one order of magnitude lower than blubber however the relationship among compounds was the same with ΣHCH < ΣCHL < ΣDDT < ΣPCB (Tables 7 and 8). The highest PCB levels in liver were two orders of magnitude lower than the highest levels in blubber (Tables 7 and 8) and nine compounds made up more than half (50.6%) of the ΣPCBs in liver. Congener 153/132 was also the dominant congener in liver and accounted for 11.5% of the ΣPCBs in liver.

Table 7. Geometric mean concentration, geometric standard deviations (SD), and ranges (ng/g or ppb wet wt) for total organochlorines in blubber from nine ribbon seals harvested in Alaska, 2003–2006.

	Compound			
	∑ HCH	**∑ CHL**	**∑ DDT**	**∑ PCB**
Mean	93.9	338.6	456.5	552.0
SD	1.64	2.10	2.06	1.94
Range	(53–228)	(199–1979)	(168–1382)	(231–1467)

Table 8. Geometric mean concentration, geometric standard deviations (SD), and ranges (ng/g or ppb wet wt) for total organochlorines in liver from eight ribbon seals harvested in Alaska, 2003–2006.

	Compound			
	∑ HCH	**∑ CHL**	**∑ DDT**	**∑ PCB**
Mean	1.9	9.6	16.1	37.0
SD	5.5	2.5	2.2	2.2
Range	(0–7)	(3–57)	(5–46)	(15–144)

Disease— Blood samples were collected from 13 ribbon seals harvested in 2003 and one harvested in 2004 near Little Diomede Island, Alaska. Serum samples were not suitable for all test procedures; thus, sample sizes differed by test. *Brucella* antibodies were found in two of 14 (14.3%) seals tested (Table 9). Seals that tested positive for *Brucella* antibodies were both females, 2 and 4 years old. All tests for antibodies of PhHV-1, PhHV-2, and PDV were negative.

Table 9. Serological results for ribbon seals sampled from Little Diomede during 2003 and 2004.

ID #	Sex	Age (yr)	*Brucella*	PDV	PhHV-1	PhHV-2
DIO-044-03	M	5	negative	negative	negative	negative
DIO-046-03	F	6	negative	negative	negative	negative
DIO-049-03	F	6	negative	negative	negative	negative
DIO-050-03	M	23	negative	unsuitable	unsuitable	unsuitable
DIO-051-03	M	7	negative	negative	negative	negative
DIO-052-03	F	2	**positive**	unsuitable	unsuitable	unsuitable
DIO-058-03	M	5	negative	negative	negative	negative
DIO-060-03	F	10	negative	negative	negative	negative
DIO-066-03	M	15	negative	negative	negative	negative
DIO-069-03	F	4	negative	negative	negative	negative
DIO-095-03	F	4	**positive**	negative	negative	negative
DIO-105-03	F	2	negative	negative	negative	negative
DIO-130-03	F	subadult	negative	negative	negative	negative
DIO-018-04	F	1	negative	unsuitable	unsuitable	unsuitable

DISCUSSION

Potential biases of harvest data

Any discussion of harvest data must address potential biases due to non-random sampling. We expect that bias due to hunter selectivity is low. Our questionnaire did not indicate that hunters avoided harvesting ribbon seals or attempted to selectively harvest any particular segment of the population. After three years of age ribbon seals have fully developed their distinctive coloration and age cannot be distinguished visually. Hence, we do not think the distribution of age classes, and having fewer seals >15 years of age in the 1960s and 1970s, is due to hunter bias.

Likewise, we don't think the large harvest of ribbon seals in 1967 was due to hunter bias. Rather, sea ice conditions that spring were unusual in that the ice edge was located several hundred miles north of normal and the ice was more fragmented than usual (Burns 1969). It appears, based upon our samples and harvest data, that subsistence hunters harvest few ribbon seals in a typical year. Kenyon (1962) reported one taken at Little Diomede in 1957, one in 1958, and stated that in some years they may harvest three or four. Only 23 ribbon seals were harvested during 1961–1966 (Burns 1969) and Burns (1985) estimated that fewer than 250 were harvested per year during 1960s–1980s. Years with high harvest are likely due to the juxtaposition of the ice front and remnant ice near coastal villages in the spring (Lowry 1985).

Many of the seals harvested in 1967 were pups, probably due to the timing of the ice and weather conditions that made the seals available for harvest, inclusion of these samples in our

analysis of age distributions would have biased the age distribution towards younger age classes in the 1960s. We removed this potential source of bias by removing pups from the analysis, thereby restricting the analysis to age classes available to hunters in all years.

Potential biases of age data

Another potential source of bias is due to the fact that ageing techniques have changed significantly during the last 40 years. Ageing seals by counting claw ridges is questionable because claws wear with age. This would bias our sample towards younger age classes in the 1960s. Burns (1969) collected both claws and teeth from ribbon seals and found a general agreement between the two methods. He believed that claws retained their ridges between 15 to 21 years, longer than that observed for other phocids, because of their more pelagic habits. When we plotted standard length versus age, the scatterplots derived with teeth and those derived with claws overlapped each other and had the same degree of variation. Furthermore, we repeated all analyses that depended upon seal age, without claw ages in the sample. Our results were virtually identical and did not affect our interpretation of the data.

Trends in population parameters

Ribbon seals are known to grow faster and mature faster than other ice seals (McLaren 1993, Burns 1986). Similar to Shustov and Yablokov (1967, as cited by McLaren 1993) and McLaren (1993), we found that growth rates did not vary by gender. McLaren (1993) estimated L_{inf} (asymptotic length) to be 163.0 (SE=1.17) for ribbon seas of the Bering Sea. Our estimate, 155.7 (SD=2.4), is similar. Because growth curve models are sensitive to the proportions of seals of different age classes, we do not think this difference is biologically meaningful.

However, we found evidence that seals grew faster in the 1970s, than in the 1960s or since 2000 (Fig. 4a, b). The higher growth rate in the 1970s may have been due to the large commercial harvests by Russian sealers (see INTRODUCTION). It is believed that the Bering Sea population consisted of 100,000 to 120,000 ribbon seals before commercial sealing began in 1961 (Fedoseev 2000). Harvest was limited in the late 1960s, by which time the population had declined to approximately 60,000 individuals (Burns 1981; citing Sustov 1972). Presumably, minimum density was reached in 1968 or 1969, when sealing quotas were reduced. Burns (1981) indicates the population may have increased by 20% between 1972 and 1974. Hence, it appears the population was recovering in the 1970s.

If resources did sometimes limit the ribbon seal population, we would expect to see the effects of density on other population parameters, such as body condition index, age distributions, and the age at first reproduction. The body condition index was significantly higher in the 1970s than the 1960s. Although the body condition index since 2000 was most similar to that from the 1960s, it is statistically indistinguishable from either the 1960s or 1970s. Relatively few seals have been collected since 2000 and we suspect that the lack of statistical significance is due to sample size limitations. This pattern corroborates the temporal pattern we observed in growth rates; conditions were more favorable for ribbon seals in the 1970s.

A larger proportion of older aged seals have been harvested since 2000 than in the 1960s or 1970s (Fig. 7). The difference in the proportion of older aged seals was only statistically significant for individuals > 20 years of age, although the same pattern was present for seals > 15 years of age. Because there were very few seals > 20 years of age in the 1960s and 1970s, this indicates that survival has increased. The most likely explanation is that a reduction in the commercial harvest has allowed seals to grow older. However, an age distribution skewed towards adults in general could also indicate a declining population. If recruitment began to fail, we would initially see a decrease in the < 5 year old age class and increases in the other age classes. However, the proportion of seals in the < 5 year old age class was similar between the 1960s (52%) and in seals sampled since 2000 (50%) (Fig. 7).

Unfortunately, we are unlikely to detect a decrease in reproduction until long after it takes place. To detect a difference in this age class would also require a large shift in the age distribution. Assuming current sample sizes, a power analysis (Proc Power in SAS v9.3, SAS Institute Inc., Cary, NC) revealed that the proportion of seals < 5 years of age would have to decrease from the current value of 50% to approximately 30% in order detect a change in age distribution between the 1960s and the current population (α=0.05, power = 80%). Because only a few seals are harvested each year, we may not detect a decline for many years.

There was also higher proportion of seals < 5 years of age in the 1970s than the 1960s or 2000s, suggesting lower density and/or more favorable conditions in the 1970s. However, this difference was not statistically significant (Fig. 7).

Evidence from the age at sexual maturity (age at first ovulation) for females is inconclusive. Given that commercial sealing began in 1961 (Burns 1986) and potentially reduced the population from ~100,000 to 80,000 by 1964 (Fedoseev 2000, citing Shustov 1969) the surviving ribbon seals were not likely to be resource limited and may have grown faster and matured at a younger age. We know that our mean ages of primiparous females are biased high relative to the true age of first ovulation, because we did not have enough data to use other methods of estimation. The range of ages for primiparous females was 1–5 years of age between 1964 and 1968, 3 years of age in a single female collected in 1978, and 2–4 years in females collected between 2003 and 2007.

During these same time periods, the percentage of pregnant females was 97.0% (32 of 33) in 1964–1968, 95.2% (20 of 21) in 1976–1979, and 91.7% (11 of 12) in 2003–2007. The decline in pregnancy rate between 1964–1968 and 2003–2007 is not statistically significant (p=0.45; chi-square w/1 df) and probably not biologically significant. Age at sexual maturity is the youngest of all of the ice seals in Alaska and the pregnancy rate is the highest (Burns 1981), making the species capable of rapid population growth, if survival is high. Although pregnancy rate is lower now than in the 1960s, it is still quite high and not likely to result in a reduction in population numbers unless survival is low.

Stomach contents

The diet of ribbon seals is poorly understood compared to other ice seals primarily because most seals stomachs are empty when harvested. Ribbon seals are available near coastal

communities during a period of reduced feeding activity (May–June) associated with reproductive activities and molt (Burns 1980, Frost and Lowry 1980). Most of what is known about ribbon seal diet in the Bering Sea comes from food items identified from the stomachs and/or intestines of 1,207 ribbon seal stomachs sampled from the central Bering Sea only 32 of which contained food (Shustov 1965). Six stomachs from St. Lawrence Island were reported by Burns (1980); four were taken in spring and two in winter. Frost and Lowry (1980) examined 61 stomachs; however, food items were only found in 28 of them. Dehn *et al.* (2007) examined 37 stomachs, but only two contained prey.

Shrimps, crabs and mysids were the most frequently identified prey items reported by Shustov (1965) in the central Bering Sea in March–July. Pollock was the dominant prey reported in the south-central and central Bering Sea and arctic cod was dominant in the northern Bering Sea in March–June (Frost and Lowry 1980). There was some evidence that diet varied with age; small crustaceans were found more often in immature ribbon seal stomachs while fish and cephalopods were found more often in adult stomachs (Shustov 1965, Frost and Lowry 1980). Examination of stable carbon and nitrogen isotopes (Dehn *et al.* 2006) also indicated that older seals foraged at higher trophic levels.

Although the additional seal stomach from Point Hope does not contribute any new prey species to the ribbon seal diet, it does provide the first diet data from the Chukchi Sea. It is possible that the fish were eaten in the Bering Sea and the otoliths were still available to be sampled in the digestive tract when the seal was harvest in the Chukchi.

Contaminants

Metals—Metals and other elements occur naturally in the marine environment and levels can vary widely in Alaska depending upon regional geology. Little is known about what the normal ranges are for marine mammals. Cadmium and mercury are commonly present at high concentrations in liver and kidney tissue of marine mammals. Cadmium can be toxic at elevated levels; however, in marine mammal kidney and liver it tends to be bound to metallothionein, which makes it less bioavailable and therefore less toxic (Goyer 1991, Groten *et al.* 1990). Marine mammals are known for their ability to use selenium to detoxify mercury and elevated mercury levels are usually accompanied by elevated selenium levels (Koeman *et al.* 1975). Most studies indicate that element concentrations generally increase with age (see review in Northern Contaminants Program 2003), yet few studies have sufficient samples to analyze for the affects of age. Alternatively, the concentrations of some elements may decline with age and some relationships may be non-linear (Dehn *et al.* 2005).

Few trace metal concentrations for ribbon seals are available for comparison. Dehn *et al.* (2006) reported Hg and Hamanaka *et al.* (1977) reported Cd and Zn in liver tissue. Six of the ribbon seals analyzed in this study were a subset of the 39 analyzed by Dehn *et al.* (2006). Our arithmetic mean Hg level of 2.10 µg/g ww (SD 6.19, range 0.41–18.06) was higher than the arithmetic mean reported by Dehn *et al.* (1.17 µg/g ww, SD 1.79, range 0.18–8.52). Our higher level is explained by the high concentration from a 13-yr-old female harvested near Point Hope in 2007 (Table 6) that was not included in Dehn *et al.* (2006).

Hamanaka *et al.* (1977) analyzed Cd and Zn in liver tissue of one 3-yr-old female and one 3–5 yr-old male ribbon seal. Their arithmetic mean Cd level was 2.57 µg/g ww (SD 0.69), which was lower than ours (6.44 µg/g ww, SD 5.78), the difference is probably due to our larger sample size. Our arithmetic means for Zn were the same (52 µg/g ww) as those reported by Hamanaka *et al.* (1977). No other data are available for comparing trace metals in ribbon seals.

Some elements are known to accumulate with age and possibly gender (*e.g.*, Dehn *et al.* 2005). The highest levels of elements of potential concern (Cd, Hg, and Pb) were found in a 13-yr-old female (Table 6). Ribbon seals had the highest mean concentration of Cd and Hg of any of the Alaskan ice seal species (ringed, bearded, spotted) that we have analyzed (ADF&G unpubl. data, Table 10). Spotted seals had the lowest levels of these elements, which is unexpected because spotted and ribbon seals are thought to share a similar distribution in the Bering Sea and have similar piscivorous diets (Frost and Lowry 1980, Bukhtiyarov *et al.*1984). Mean Pb levels were very low and similar among species (Table 10).

Table 10. Geometric mean concentration, geometric standard deviations (SD), and ranges (µg/g or ppm wet wt) for potential metals of concern in liver from ice seals harvested in Alaska 2003–2007. The highest concentration for each metal is in bold.

Metal		**Species**			
		Ringed	**Bearded**	**Spotted**	**Ribbon**
	n	32	38	17	9
Cd	Mean	1.59	2.28	0.38	**3.64**
	SD	2.86	7.12	3.98	**3.65**
	Range	(0.17-20.80)	(0.01-39.93)	(0.02-6.40)	**(0.42-15.21)**
Hg	Mean	1.21	1.91	0.88	**2.10**
	SD	3.20	3.69	4.16	**4.10**
	Range	(0.14-12.88)	(0.13-28.31)	(0.03-5.61)	**(0.41-18.06)**
Pb	Mean	0.04	0.04	0.04	0.04
	SD	1.57	1.71	1.67	1.68
	Range	(0.03-0.12)	(0.03-0.48)	(0.03-0.22)	(0.03-0.13)

Our mean Hg (2.10 µg/g ww, SD 4.10) and Cd (3.64 µg/g ww, SD 3.65) levels in liver were lower than those reported by Riget *et al.* (2005) for ringed seals from Barrow, Alaska (Hg = 3.52 µg/g ww, SD 5.07; Cd = 5.72, SD 3.21), Canada (Hg mean range 9.4–31.9, SD 15.0–58.9; Cd mean range 2.73–12.5, SD 2.96–9.18) , and Greenland (Hg mean range 1.40–6.22, SD 2.50–5.54; Cd mean range 8.48–13.0; SD 7.29–13.1). Our mean Hg level was higher than Svalbard (0.97, SD 0.65) and the White Sea (2.25, SD 2.14), but our mean Cd levels were similar (Svalbard 3.90, SD 3.59; White Sea 2.56, SD 3.02) (Riget *et al.* 2005)

Organochlorines—Compared to other ice seals in Alaska (ADF&G, unpubl. data), ribbon seals had the highest geometric mean concentrations of ΣCHL (357.8 ng/g lipid wt), ΣDDT

(446.6 ng/g lipid wt), and ΣPCB (547.8 ng/g lipid wt) in blubber tissue (Table 11). However, spotted seals had the higher ΣHCH levels (103.0 ng/g lipid wt) than ribbon seals (93.9 ng/g lipid wt).

The finding that DDE was the dominant compound detected indicates that ribbon seals in Alaska have not been exposed to recent applications of DDT. DDE is formed as DDT degrades. Because ribbon seals are more common along the Russian than the Alaskan coast, if DDT were still commonly used we would expect to find higher levels of DDT rather than DDE in ribbon seal tissues.

Although ribbon seals had higher levels of OCs than other ice seal species in Alaska the levels are generally lower than for ringed seals in Canada (*e.g.*, Muir et al. 1999). However, comparison of studies can be problematic. First, studies often examine different OC congeners, making direct comparisons impossible. Second, concentrations may depend both on gender and age, so differences in mean concentrations may have more to do with the sex ratio of the seals sampled than the location or time period.

Table 11. Geometric mean concentration, geometric standard deviations (SD), and ranges (ng/g or ppb wet wt) for total organochlorines in blubber from ice seals harvested in Alaska 2003–2006. The highest concentration for each metal is in bold.

Compound		**Species**			
		Ringed	**Bearded**	**Spotted**	**Ribbon**
	n	32	33	17	9
∑ HCH	Mean	51.8	14.4	**104.8**	93.9
	SD	1.65	1.55	**1.56**	1.64
	Range	(17-150)	(3-28)	**(35-313)**	(53-228)
∑ CHL	Mean	96.5	104.2	193.6	**338.6**
	SD	2.12	1.60	1.96	**2.10**
	Range	(24-342)	(51-415)	(38-580)	**(199-1979)**
∑ DDT	Mean	129.3	91.2	199.5	**456.5**
	SD	1.85	1.95	2.19	**2.06**
	Range	(39-628)	(26-605)	(30-695)	**(168-1382)**
∑ PCB	Mean	278.7	193.0	404.1	**552.0**
	SD	1.71	1.76	1.97	**1.94**
	Range	(92-908)	(69-943)	(99-1256)	**(231-1467)**

Other Contaminants—Polybrominated diphenyl ethers (PBDEs) have been analyzed in the blubber of ribbon seals (Quakenbush 2007). PBDEs are chemicals widely used as flame retardant additives in carpets and upholstery, and in plastics used in electrical appliances, televisions, and computers. It is thought that PBDEs enter the food chain by being released

slowly into the air through the life of the products that contain them (Strandberg *et al.* 2001). Although little is known about the toxicology of PBDEs, PBDEs and their congeners are structurally similar to polychlorinated biphenyls (PCBs) and thyroid hormones. Lab studies indicate that PBDEs may disrupt thyroid function and neurodevelopment (Darnerud 2003, Viberg et al. 2004). Ribbon seals had the highest mean level of total PBDEs (16.5 ng/g wet wt) compared to other Alaska seal species, but had lower levels compared to seals from other regions of the Arctic (Quakenbush 2007).

Perfluorinated contaminants (PFCs) have been analyzed in the liver of ribbon seals (Quakenbush and Citta 2008). PFCs affect cellular function and intercellular communication; however, the concentrations at which PFCs become toxic to seals are unknown. PFCs are not lipophilic like OCs, instead they are lipophobic, and the way they are acquired and how they bioaccumulate are not known. Perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA) were detected in most samples (Quakenbush and Citta 2008). When compared to other Alaska seals, ribbon seals had similar concentrations of PFOS and PFNA but they had the highest levels of perfluoroundecanoic acids (PFUnDA). PFOS has been identified as the predominant PFC in wildlife. Studies of ringed seals in Canada (Martin *et al.*2004) and Greenland (Bossi *et al.*2005) generally find levels of PFOS twice as large as what is observed in ribbon seals (Quakenbush and Citta 2008). However, they found larger mean levels of PFNA, PFDA and PFUnDA. Because little is known about the transport mechanism, the way the different compounds are acquired, and how they affect seals we have no explanation for why concentrations are different in ribbons seals or whether they are harmful.

Genetics

Our investigation of stock structure using mtDNA did not reveal any separate stocks, but found a high diversity of haplotypes. This does not necessarily mean that there is no stock structure in the population. First, our samples were collected after the breeding season when ribbon seals are becoming pelagic. During this time they are likely moving great distances from their breeding areas and different stocks might be mixing. Second, detecting stocks might be easier with microsatellite DNA than mtDNA. Microsatellite DNA is thought to be under little selection pressure and, therefore, evolves more quickly than mtDNA. Hence, microsatellite DNA should provide a more detailed assessment of stock structure. We plan to reanalyze our samples using microsatellite DNA techniques.

Disease

We identified Brucella antibodies in 14.3% (2 of 14) of our samples. In general, low levels of *Brucella* have been found in Arctic species. Nielsen *et al.* (1996) identified *Brucella* antibodies in 4.0% (10 of 248) of ringed seals in the Canadian Arctic and Tryland *et al.* (2001) identified *Brucella* antibodies in 5.4% (16 of 297) of polar bears near Svalbard. In contrast, Zarnke *et al.* (2006) found high incidence, 46.0% (46/100), of *Brucella* antibodies in harbor seals from the Gulf of Alaska, similar to incidence rates, 49.0% (147/300), observed in harbor seals from Scotland (Foster *et al.* 2002). Harbor seals experience closer contact with one another on their terrestrial haulouts than ribbon seals, ringed seals, or polar bears do on sea ice and this may explain the higher prevalence in them. However, it is difficult to determine how the prevalence

of *Brucella* antibodies in ribbon seals compares to these other species, because our sample size is low. All we can safely conclude is that *Brucella* is present in ribbon seals.

Neither mortality, nor reproductive disorders were noted in any of the studies cited above and it is believed that Brucellosis is not a significant source of reproductive failure in seals. However, Foster *et al.* (2002) notes there is little or no data on abortion rates, so Brucellosis may be more important than what is currently assumed.

PhHV-1 was first identified in 1984, when it caused the deaths of 11 harbor seal pups in the Netherlands (Osterhaus *et al.*1985). Symptoms include fever, vomiting, and diarrhea (Visser *et al.* 1991). Colegrove *et al.* (2005) sampled live stranded harbor seals in California and found that 3-6% of live strandings were primarily or secondarily attributable to PhHV-1, although in some years PhHV-1 was responsible for 10-20% of strandings. PhHV-2 has been detected in harbor seals from the North Atlantic (Harder *et al.* 1996) and the North Sea (Lebich *et al.* 1994). We detected no PhHV-1 or PhHV-2 antibodies in ribbon seal serum. This is a curious result because Zarnke *et al.* (1997) identified antibodies of both in 29.2% (7 of 24) of ribbon seals sampled in the Bering Sea. Although sample sizes are small, there is only a 2.5% probability of not detecting PhHV-1 or -2, if incidence rates have remained the same and if we are really sampling one population or stock (chi-square test w/1 df). Although our genetic study using mtDNA did not reveal any stock structure, stock structure of ribbon seals may exist. More disease screening is necessary to verify the prevalence of PhHV-1 and -2.

It is also interesting that we did not detect antibodies for PDV. It is thought that PDV is circulating within Arctic species (*e.g.*, Barrett *et al.* 1995, Duignan *et al.* 1997, Härkönen *et al.* 2006) and that harp seals (*Phoca groenlandica*) may be the major reservoir for PDV in the Arctic (Barrett *et al.* 1995, Duignan *et al.* 1997). Harp seals have a high prevalence of PDV antibodies (83%; 130 of 157) (Duignan *et al.* 1997) and exhibit attributes conducive for maintaining a virus, such as a large population size and dense aggregations. Duignan *et al.* (1997) also found that ringed seals had a high prevalence rate (41%; 106 of 259), which is surprising given their dispersed population structure. The prevalence of antibodies was highest where ringed seal and harp seals overlap in range, supporting the idea that harp seals might serve as a reservoir. Harp seals are also believed to be the source of the 1998 PDV outbreak in the northern Europe (Heide-Jørgensen *et al.* 1992, Härkönen *et al.* 2006). Migrating harp seals have been observed in the North Atlantic and they are believed to have transferred PDV into grey seals (*Halichoerus grypus*), which are largely immune (Barrett *et al.* 1995, Härkönen *et al.* 2006, Heide-Jørgensen *et al.* 1992). PDV has been documented as persisting within grey seal populations (Barrett *et al.* 1995, Hammond *et al.* 2005) and both the 1988 and 2002 outbreaks of PDV in harbor seals have been traced to a single haulout in Denmark (Anholt). This haulout is notable in that both grey and harbor seals haulout together and mix (Härkönen *et al.* 2006).

The range of ribbon seals in the Bering Sea overlaps that of both ringed and harbor seals. We have detected PDV antibodies in ringed seals (ADF&G unpublished data) and Zarnke *et al.* (2006) found a 1% (68 of 191) prevalence rate of PDV antibodies in harbor seals within the Gulf of Alaska. However, exposure does not guarantee an epizootic. Although PDV results in high mortality rates in harbor seals (an outbreak of PDV in northern Europe killed over 23,000 harbor seals in 1998 and 30,000 in 2002; Härkönen *et al.* 2006), other phocids are largely immune to

PDV. For example, grey seals are much less susceptible to PDV (Barrett *et al.* 1995, Härkönen *et al.* 2006) than harbor seals, only one harp seal has shown clinical disease attributed to PDV (Daoust *et al.* 1993), and there are no cases of clinical disease in ringed seals. Transmission rates are also affected by seal behavior, which changes seasonally. Ribbon seals loosely aggregate into disjoint breeding herds in the Bering Sea, along the ice front in April and May (Fedoseev 2000). This is likely when they would be most susceptible to an epidemic. We doubt an epizootic could form during the summer and early fall, when ribbon seals are largely pelagic.

A disease threat we did not examine is canine distemper virus (CDV). An outbreak of CDV killed thousands of Baikal seals (*Phoca sibirica*) in 1988 (Grachev *et al.*1989, Mamaev *et al.* 1995) and over 10,000 Caspian seals (*Phoca caspica*) in 2000 (Kennedy *et al.* 2000). Both epidemics are believed to have been caused by seals coming into contact with terrestrial carnivores that were disease vectors. Given that ribbon seals rarely haulout on shorefast ice it is not likely that they will come in contact with terrestrial carnivores, however, CDV is the dominant morbillivirus in polar bears. Follmann *et al.* (1996) found morbillivirus antibodies in 35% (68 of 191) of polar bears from Alaska and Russia. These were later identified as antibodies for CDV (Garner *et al.* 2000). When ribbon seals are hauled out on pack ice they could come into contact with arctic foxes and polar bears carrying CDV. It is also possible that CDV could spread from polar bears to ringed seals and then into ribbon seals. Again, transmission rates would depend upon the concentration of ribbon seals and the connectivity between groups, information which is largely unknown.

CONCLUSION

Population status—There is no evidence to suggest that ribbon seal growth rate, body condition, age at first reproduction, or productivity currently differs from what was observed in the 1960s. Growth rates and body condition indices were higher in the 1970s than the 1960s or since 2000. We suspect that this may be in response to large commercial harvest by Russian sealers; based upon descriptions of Burns (1986) and Fedoseev (2000), the population was most depressed in 1967 or 1968. Hence, the population would have been recovering throughout the 1970s. However, conditions may have simply been more favorable in the 1970s. The commercial harvest might also be why there were fewer seals greater than 15–20 years of age in the 1960s and 1970s.

Unfortunately, a trend towards older age classes in the population may also be indicative of a declining population. Ribbon seals depend upon sea ice for pupping and nursing; if sea ice were limited during this period, we may expect pup survival to be a primary mechanism of population decline. Although pregnancy rates and the age at first reproduction have not changed, we have no information on pup survival therefore, we cannot rule out the possibility that a shift towards older animals in the harvest could be the first signs of a declining population. For this to be true however, sea ice would have to be limiting during pupping. We do not believe that sea ice is currently limiting; however, evaluating pup survival is critical to addressing that issue.

If climate change is affecting the ribbon seal population, it has yet to affect other indices of population status that we have data for. Unfortunately, few ribbon seals are typically

harvested by subsistence hunters in Alaska. As such, only one or two samples are collected in a typical year allowing us limited ability to detect changes in the population.

Contaminants—Although ribbon seals in Alaska have higher levels of most metals, OCs, and other contaminants such as PBDEs and PFCs than other Alaskan species, their levels are not higher than ringed seals in the Arctic in most cases. The effects of contaminants on seals are difficult to evaluate because of their ability to detoxify some and biotransform or excrete others. Therefore, a high level of Hg measured in a tissue may not mean there is any toxic affect to the seal. OCs levels generally increase with animal age for males, but not females because females may transfer OCs through the placenta or via lactation decreasing their own levels once they become reproductive. Accounting for age effects are important when different age distributions are sampled. Most contaminant studies compare levels within species, among regions, and through time to look at trends, but there is little information on what the levels mean relative to the seals' health.

Disease—We detected low prevalence of *Brucella* antibodies (14.3%: 2 of 14) and detected no antibodies for PhHV-1, PhHV-2, or PDV. Based upon other studies, we doubt *Brucella*, PhHV-1, or PhHV-2 will pose serious threats to population persistence. PDV is documented as having caused large epidemics in harbor seals. The effect of PDV spreading into the population of ribbon seals is difficult to predict, as ribbon seals may be largely immune like harp, ringed, or grey seals. If ribbon seals are susceptible to PDV, then the effect on the population will vary seasonally. Ribbon seals would be most susceptible during pupping, breeding, and molting, when they are loosely aggregated along the ice front. We think an epidemic would be unlikely during the summer or fall, while ribbon seals are pelagic.

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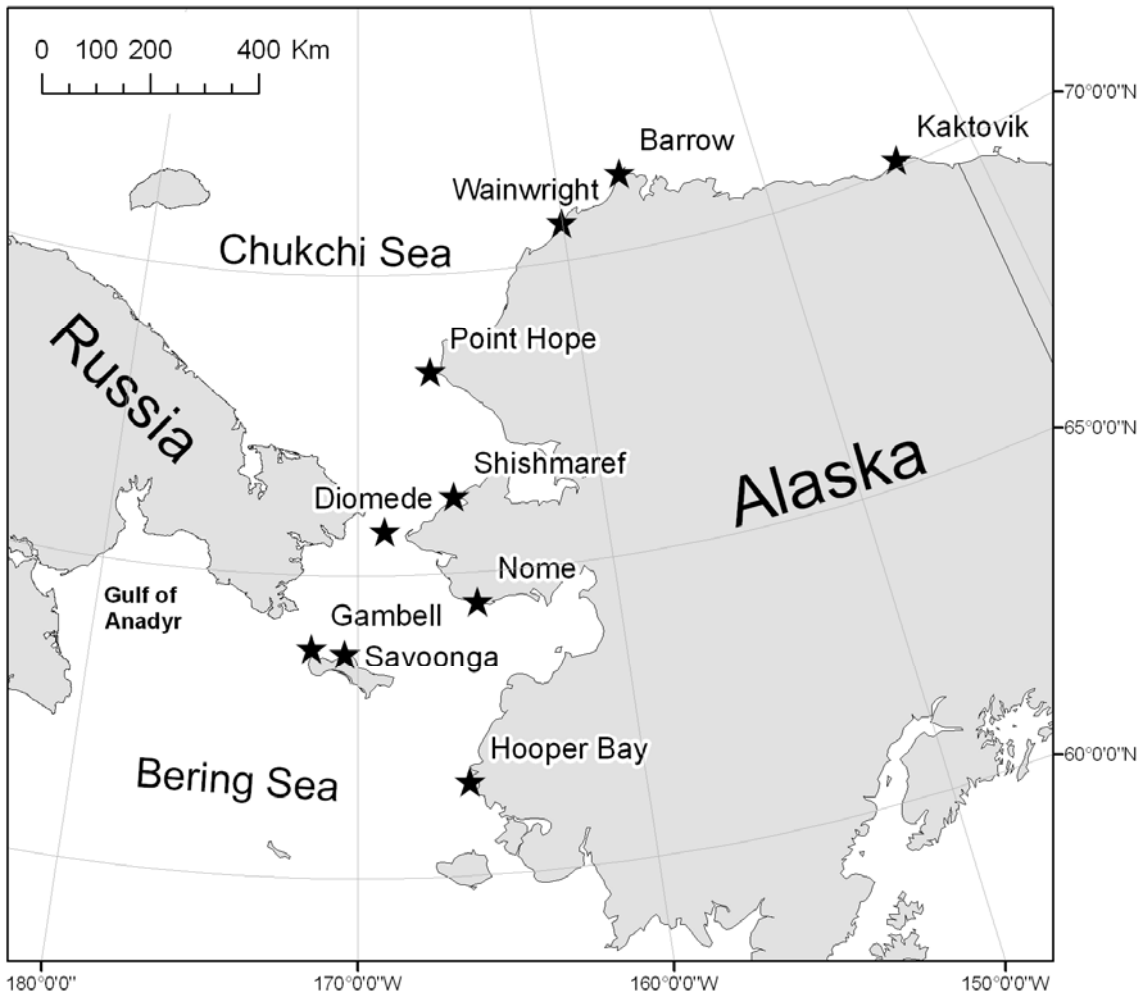

Figure 1. Sampling locations in Alaskan waters.

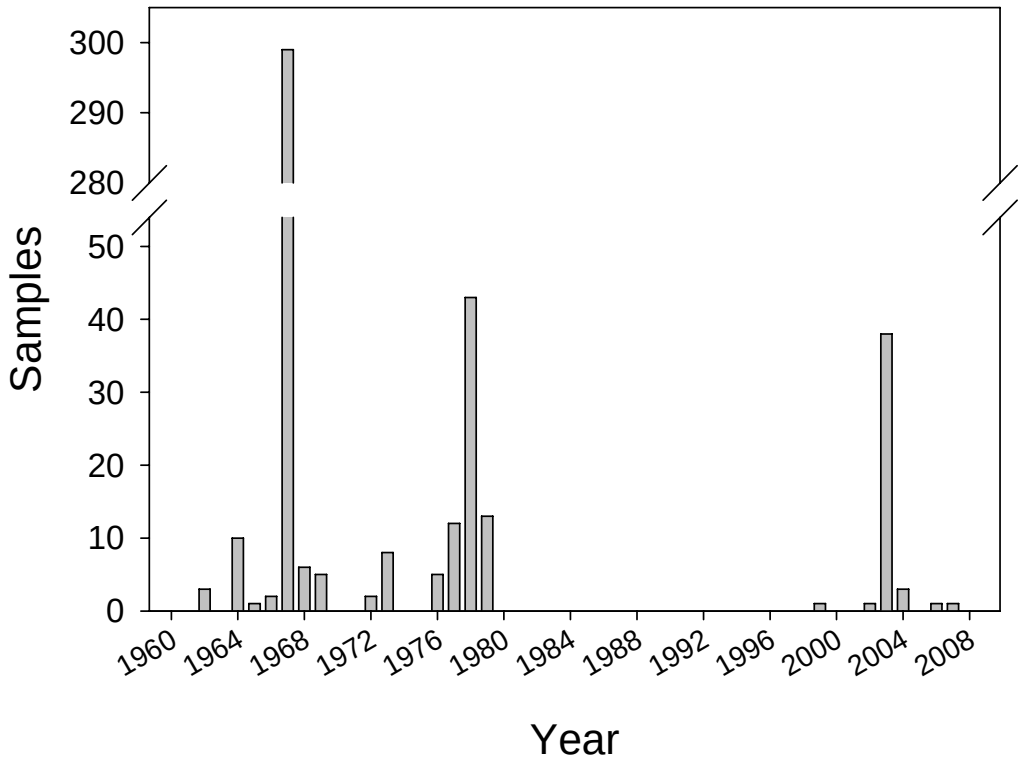

Figure 2. Number of ribbon seals collected by year in Alaska for which ages are known. The sample size of seals differed slightly for each analysis, as not all measurements or samples were collected for each seal. Although sample sizes differ by analysis, this figure illustrates the distribution of samples by year.

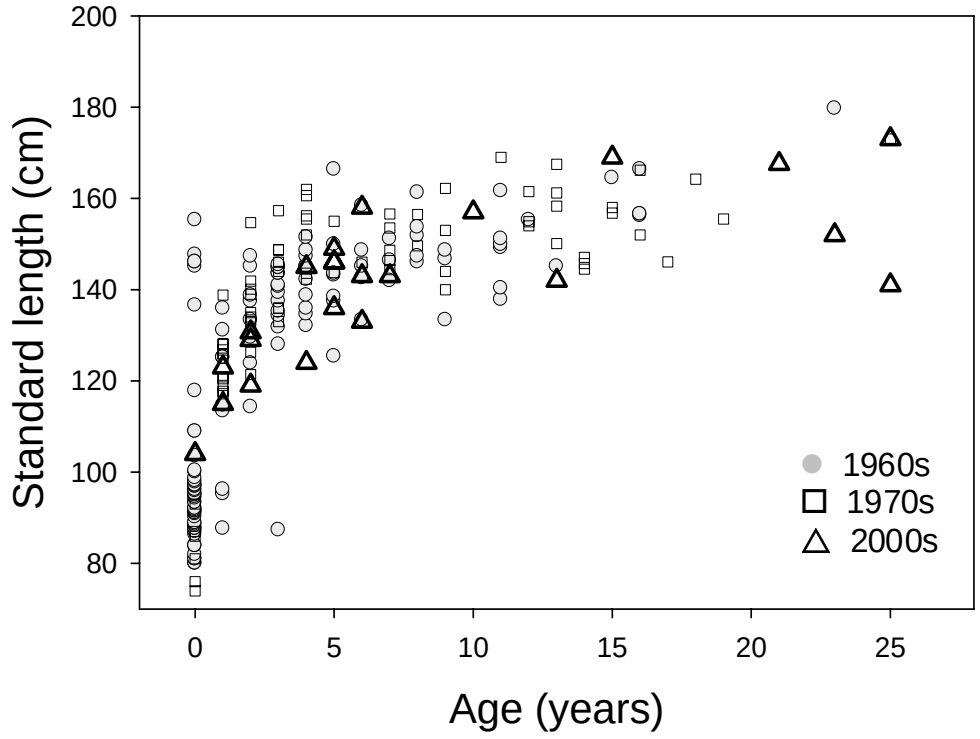

Figure 3. Plot of length-at-age for ribbon seals collected during three time periods (1960s, 1970s, and since 2000).

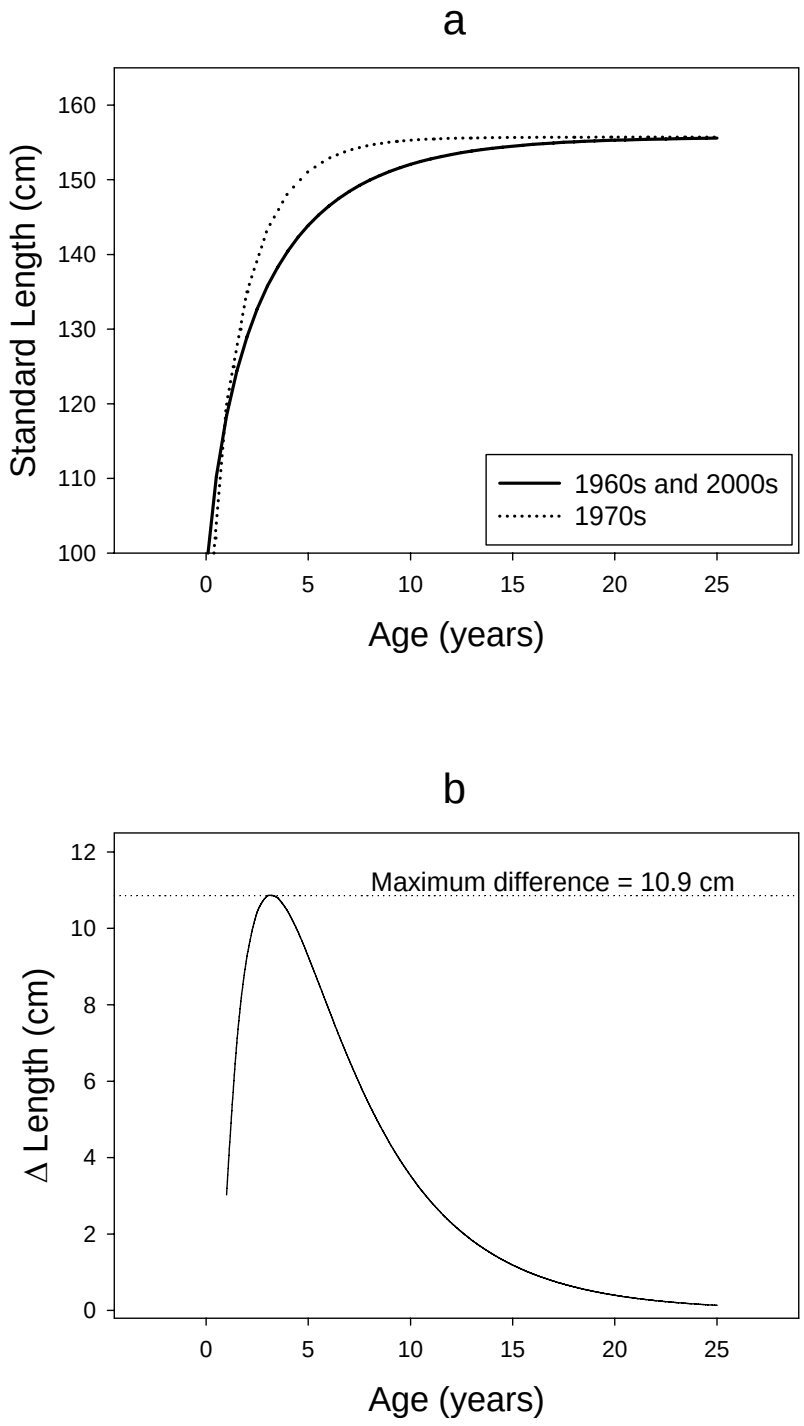

Figure 4. a) Mean predictions of length-at-age from the best approximating model of ribbon seal growth; b) Plot of the average difference in length between seals in the 1970s and the 1960s or 2000s.

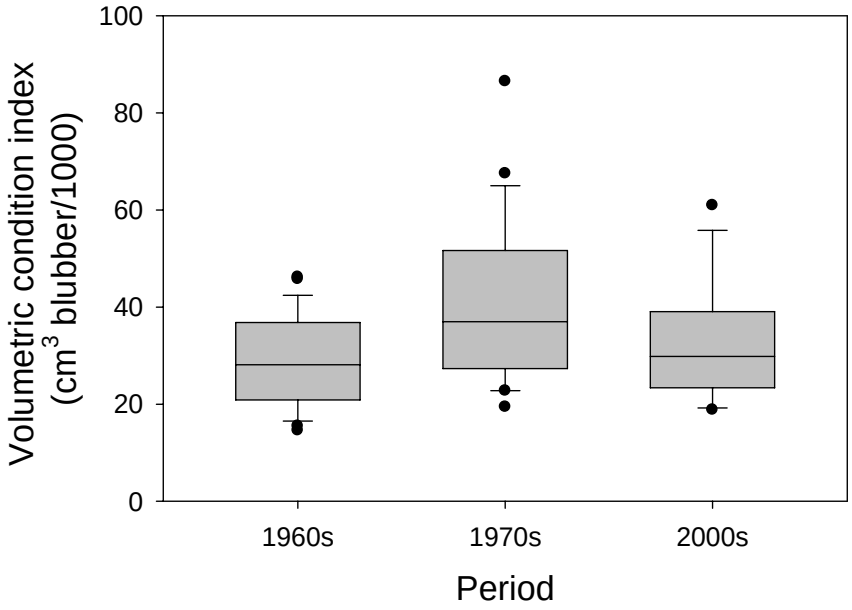

Figure 5. Box plots of the volumetric index of body condition; boxes define the 10th and 90th percentiles, ears are the 95th percentiles, and dots represent each outlier. Non-parametric rank tests show that seals collected in the 1960s had a lower mean value than seals in the 1970s (p=0.03). Seals collected since 2000 were most similar to those collected in the 1960s, but were statistically indistinguishable from those collected in the 1970s (p=0.19).

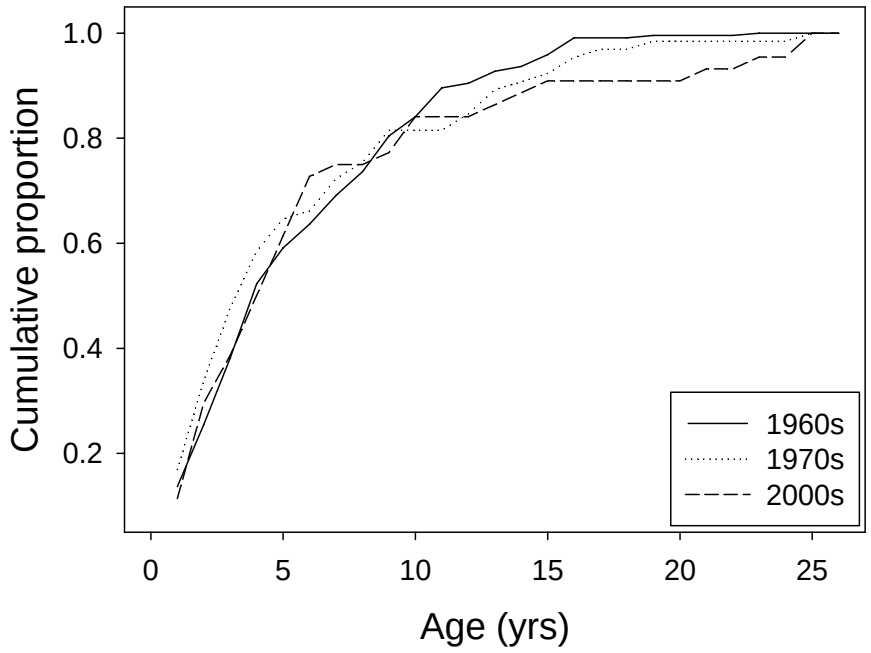

Figure 6. Cumulative proportions of ribbon seals by age, excluding pups.

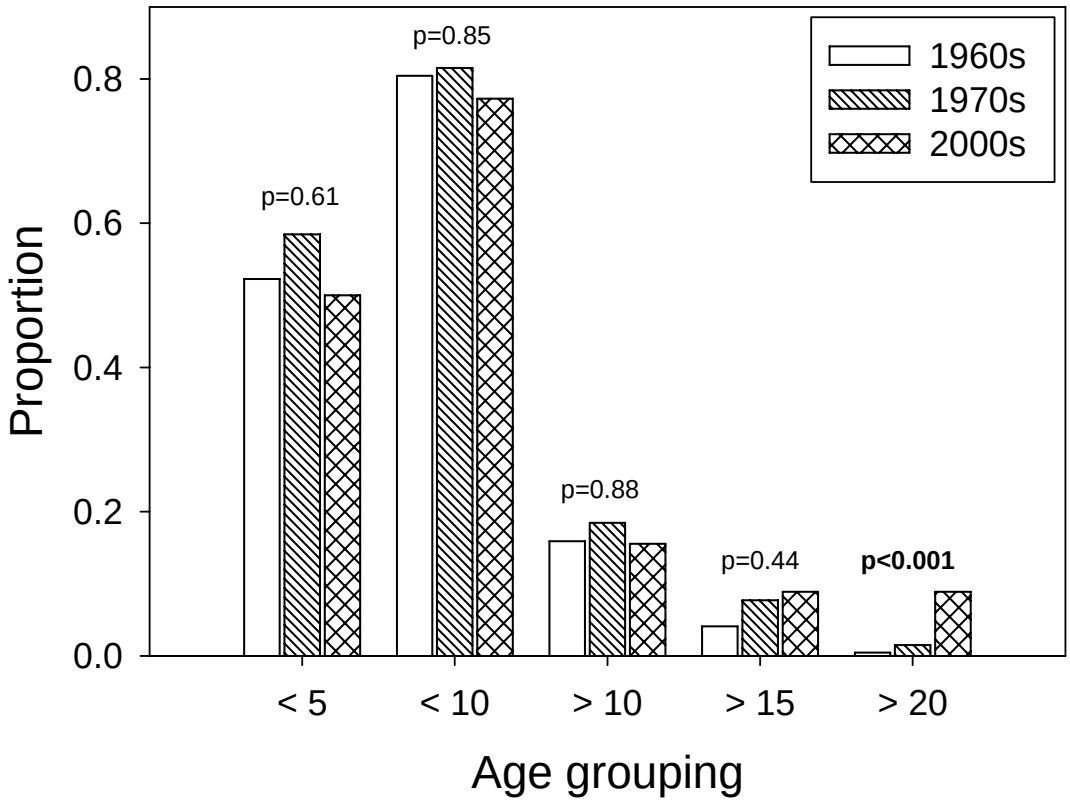

Figure 7. Proportions of ribbon seals within five different age categories and three time periods, excluding pups. Probabilities were calculated with chi-square tests.

APPENDIX

Concentrations of 82 PCB congeners in blubber tissue of nine ribbon seals collected from the Bering Sea between 2003 and 2007. Six seals were harvested near Little Diomede, two near Point Hope, and one near Hooper Bay (Fig. 1).

Individual Gender/Age (yrs)		**F/6**	**F/2**	**F/3**	**F/2**	**F/13**	**M/05**	**M/2**	**M/9**	**M/21**
Lipid (%)		93.08	94.39	91.74	89.29	88.12	86.79	93.59	88.16	91.90
PCB cpd	**MDL**									
1	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7/9	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8/5	0.19	0.00	0.00	0.00	0.00	4.41	0.00	0.00	0.00	0.00
15	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16/32	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.13	0.32	0.44	0.37	0.47	1.16	0.24	0.39	0.40	0.00
22/51	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24/27	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	0.13	0.00	0.00	0.00	0.00	2.31	0.00	0.00	0.00	0.00
28	0.14	2.24	4.07	4.22	0.00	2.26	1.58	2.32	3.12	0.94
29	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31	0.13	0.00	2.27	2.94	0.00	3.74	0.00	0.00	2.46	0.00
33/53/20	0.13	0.51	1.18	2.01	0.00	1.47	1.02	0.81	1.42	0.00
40	0.12	0.00	0.00	0.00	0.74	0.00	0.00	0.00	0.00	0.46
41/64	0.12	0.00	2.35	2.55	6.57	0.00	0.00	0.00	0.00	0.00
42/59/37	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
43	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
44	0.07	0.00	0.00	0.00	4.58	4.83	0.00	0.00	0.00	0.25
45	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
46	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47/48/75	0.12	3.30	5.84	18.19	11.07	14.77	4.07	4.57	15.25	0.00
49	0.12	1.69	4.76	10.86	6.23	9.38	2.52	2.82	7.26	3.36
52	0.12	9.92	18.82	28.35	21.07	17.86	11.00	12.77	15.46	11.20
56/60	0.12	0.00	1.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00
66	0.10	0.00	0.00	0.00	1.08	3.78	0.00	0.00	0.00	0.00
70	0.12	4.28	11.41	14.81	0.00	0.00	5.09	6.01	11.32	0.00
74/61	0.12	6.25	10.20	20.46	19.86	7.83	6.54	8.35	19.96	13.98
77	0.12	--	--	--	0.00	0.00	--	--	--	0.00
81	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
82	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
83	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
84	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
85	0.09	8.38	10.97	16.77	15.90	12.11	7.84	10.28	30.70	24.84
86	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
87/115	0.12	5.28	7.53	13.30	7.73	9.98	5.31	4.65	12.72	5.48
88	0.09	0.00	2.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92	0.10	12.12	16.52	22.60	0.00	0.00	14.19	15.79	20.35	0.00

Appendix. Continued.

Individual Gender/Age (yrs)		**F/6**	**F/2**	**F/3**	**F/2**	**F/13**	**M/05**	**M/2**	**M/9**	**M/21**
Lipid (%)		93.08	94.39	91.74	89.29	88.12	86.79	93.59	88.16	91.90
PCB cpd	MDL									
95	0.11	0.00	0.00	0.00	0.00	8.63	0.00	0.00	7.65	2.55
97	0.12	2.67	4.01	0.00	0.00	9.25	0.00	0.00	6.36	0.95
99	0.13	29.31	29.74	54.61	58.84	32.04	25.11	33.00	148.71	121.31
101/90	0.14	47.04	51.96	89.30	64.86	103.76	32.34	45.07	309.01	275.54
105	0.16	2.01	2.67	3.72	6.95	5.42	1.47	2.28	2.41	11.19
107	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
110/77	0.10	3.36	5.57	8.42	7.90	7.58	3.19	4.85	5.39	3.90
114/131/122	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
118	0.13	10.06	14.48	30.79	27.16	16.47	5.58	8.99	20.19	8.47
126	0.15	--	--	--	0.00	0.00	--	--	--	0.00
128	0.15	5.78	6.42	10.49	16.87	7.67	0.00	7.83	28.38	15.82
129/126	0.17	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
136	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
138/160	0.17	36.66	31.67	60.76	120.01	57.19	25.31	34.20	174.02	211.53
141/179	0.17	0.00	0.00	0.00	7.22	0.00	0.00	0.00	0.00	1.89
146	0.17	9.93	11.20	18.27	24.09	15.40	9.06	10.62	41.88	47.40
149/123	0.17	2.73	4.93	4.99	4.79	10.01	2.22	2.43	2.24	7.84
151	0.17	3.32	4.86	8.45	0.00	0.00	2.86	3.94	0.00	7.20
153/132	0.12	75.20	60.02	107.80	142.10	97.37	41.91	58.05	387.97	390.44
156/171/202	0.17	2.42	2.16	3.83	4.88	5.73	1.57	2.18	10.67	13.15
158	0.17	4.86	9.36	8.09	6.52	4.02	6.25	6.65	9.33	9.01
166	0.17	0.00	0.00	0.00	0.00	0.90	0.00	0.00	0.00	0.14
167	0.17	0.00	0.26	1.88	0.00	0.00	0.00	1.26	0.00	0.00
169	0.09	--	--	--	0.00	0.00	--	--	--	0.00
170/190	0.18	4.46	4.56	7.13	10.76	10.44	3.47	4.97	23.57	23.68
172	0.12	0.94	0.98	1.29	1.22	2.24	0.55	0.87	2.80	4.97
174	0.12	0.44	0.88	1.16	0.00	2.48	0.47	0.90	0.96	1.10
176/137	0.12	0.00	0.00	8.68	0.00	0.00	0.00	0.00	12.35	9.55
177	0.12	1.67	1.66	2.27	3.77	4.88	0.00	0.00	4.70	7.90
178	0.12	1.67	1.41	0.00	2.56	4.13	0.78	1.67	4.66	10.61
180	0.12	8.33	6.51	12.70	20.01	21.53	3.54	5.93	64.06	69.98
183	0.12	0.00	0.00	0.00	73.23	28.14	0.00	0.00	19.72	28.51
185	0.12	0.00	0.00	0.00	0.69	0.00	0.00	0.00	0.93	0.00
187	0.11	7.76	7.79	10.27	14.71	23.17	3.90	5.50	20.79	59.10
189	0.12	1.61	0.00	13.59	0.00	0.00	0.00	0.00	1.66	0.00
191	0.12	0.15	0.00	0.11	0.00	0.12	0.00	0.00	0.49	0.56
194	0.09	0.54	0.29	0.62	1.07	1.77	0.17	0.25	3.24	5.49
195/208	0.09	0.00	0.00	0.00	0.56	1.03	0.00	0.00	0.00	0.00
196/203	0.09	1.13	0.77	2.11	2.25	3.08	0.46	0.64	5.51	6.98
199	0.09	--	--	--	2.65	4.47	--	--	--	--
200	0.09	--	--	--	0.00	0.00	--	--	--	--

Appendix. Continued.

Individual Gender/Age (yrs)		**F/6**	**F/2**	**F/3**	**F/2**	**F/13**	**M/05**	**M/2**	**M/9**	**M/21**
Lipid (%)		93.08	94.39	91.74	89.29	88.12	86.79	93.59	88.16	91.90
PCB cpd	**MDL**									
201	0.09	1.70	1.32	1.56	--	--	1.12	1.13	5.14	9.74
200/157/173	0.08	0.26	0.00	0.49	--	--	0.00	0.00	0.00	0.00
201/157/173	0.09	--	--	--	0.00	1.17	--	--	--	--
205	0.09	0.00	0.00	0.00	0.12	0.18	0.00	0.00	0.30	0.42
206	0.08	0.22	0.22	0.44	0.62	1.30	0.11	0.33	1.39	3.24
209	0.09	0.12	0.00	0.00	0.07	0.58	0.00	0.00	0.45	1.11
Total PCB		**320.72**	**365.93**	**631.27**	**721.77**	**588.06**	**230.81**	**312.30**	**1467.37**	**1431.83**