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Biological specimen banking in Arctic research: an Alaska perspective

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ABSTRACT

The cryogenic archival of biological specimens for retrospective analysis is of significant value for present and future research on population genetics, pathology, systematics, toxicology and environmental monitoring. This realization is emphasized by the increasing support of this activity by various government agencies, institutions and international groups. The international Arctic community is no exception. Canada has been conducting such activities in association with environmental monitoring programs for many years. Similar efforts appear to be underway in other polar nations. From the perspective of the United States Arctic, the Alaska Marine Mammal Tissue Archival Project (AMMTAP) was the earliest organized effort to develop an environmental specimen bank specifically designed for long-term archival of biological specimens under cryogenic conditions. The AMMTAP emphasizes use of standardized rigorous sampling and archival protocols, procedures that minimize contamination of samples during collection and maintaining a detailed record of sample history. The development of this specimen bank, recent activities of this project and other cryogenic specimen banks being developed in Alaska are described.

Key words: biological specimen banking; Alaska; Arctic research; AMMTAP

INTRODUCTION

Within the last 30 years, the development of cryogenic preservation techniques coupled with increased emphasis on biological research at the molecular level has elevated the visibility of biological specimen banking as a routine and important part of research on systematics, genetics, pathology, toxicology and environmental monitoring. There appears to be increasing support for this activity by various government agencies, institutions and international groups. The international Arctic scientific community is no

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exception. The Arctic is becoming an arena for international science that transcends national boundaries and interests. With a growing appreciation for the importance of Arctic ecosystems, an international effort to protect the Arctic environment and to develop comprehensive monitoring is underway. The updated draft proposal for the international Arctic Monitoring and Assessment Program recommends that the archival of biological specimens for retrospective analysis be part of the Quality Assurance procedures for environmental monitoring in the Arctic [1].

Since the mid-1980s, emphasis on Arctic research in the United States has increased, particularly regarding the health of animal populations and their habitats and environmental problems that might result from increased resource exploitation and industrialization of this region. That the role of biological specimen banking in this research has been recognized is illustrated by the examples and descriptions of Arctic resource specimen banks presented in this paper.

ENVIRONMENTAL CONCERNS IN ALASKA AND THE ARCTIC

Alaska is usually thought of as a pristine wilderness, one of the last frontiers, largely untouched by modern urbanization and industrial development and the associated environmental problems. Although such is generally the case, Alaska does have a long history of natural resource exploitation (sealing and sea otter hunting in the 18th–19th centuries, whaling, commercial fishing and mining in the 19th–20th centuries, timbering and petroleum production in the 20th century). Communication and routine travel between the 48 contiguous states and Alaska increased substantially after World War II. This was followed by increased oil and gas exploration and development in the State, which climaxed with the development of the Prudhoe Bay oil field, the construction of the Trans Alaska Pipeline, the construction of the Prince William Sound oil tanker terminal and the associated urbanization and industrialization of some regions of the State (e.g. Anchorage, Fairbanks, Kenai Peninsula, Prudhoe Bay).

Presently, local sources of environmental contaminants are limited in Alaska. Sources are localized and widely scattered but they probably do contribute to the presence of polycyclic aromatic hydrocarbons (PAHs), heterocyclics, polychlorinated biphenyls (PCBs) and related compounds and organometallic compounds (organo-mercury, organo-tin) in the Alaska marine ecosystem.

Although the petroleum industry continues to be the driving economic force in the State, mining (coal, gold, silver, platinum, lead, zinc, copper, molybdenum) appears to be recovering from an extended period of depres-

sion. The redevelopment of this industry, which extends across the State from the Arctic to the Southeastern Panhandle, has the potential for creating environmental problems if not handled correctly.

Beginning in World War II and continuing through the 'Cold War', Alaska has been of sufficient strategic significance that a large military presence has been maintained there. From military bases in the Aleutian Islands and the central and southcentral part of the State to DEW (Distant Early Warning) line sites across the Arctic, installations established and maintained for over 40 years are potential sources of toxic compounds (PCBs, pesticides, toxins associated with munitions, etc.)

Industrial and agricultural sources from lower latitudes appear to be very important in introducing persistent toxic substances to the Arctic. Transport mechanisms for contaminants from lower latitudes probably involve a combination of riverine input and oceanic/atmospheric mediated transport (Fig. 1) [2–10].

The Arctic Ocean is sometimes conceptualized as a 'Polar Mediterranean', that is a sea with limited water exchange with the rest of the world's oceans [9). Circumpolar runoffs from adjacent continents converge into the Arctic Ocean. The Soviet Yenisei, Lena and Ob rivers contribute over 80% of the total river discharge to the Arctic Basin while the North America Mackenzie River contributes about 15% [4]. It is possible that contaminants originating from the circumpolar drainage basins may remain in the Arctic surface water where they can be distributed throughout the basin.

The greatest exchange of water between the Arctic Ocean and the rest of the world's oceans is with the North Atlantic through Fram Strait. Coming from the direction of offshore America and Western Europe, this inflow extends to the sea surface and has the potential for transporting agrochemical and industrial pollutants from these geographical sources into the Arctic. In time, the Atlantic water is found in all parts of the Polar Basin.

Although river discharges and oceanic circulation are important mechanisms to consider regarding contaminant transport, it appears that the atmosphere might be the major pathway for transporting semi-volatile compounds (e.g. PCBs and chlorinated pesticides) to the Arctic [11–14]. Important sources of atmospherically transported pollutants appear to be industrialized regions of Europe and North America and agricultural areas of Eastern Europe, Central Asia and the southern United States [5,15]. Even in countries where application of certain chlorinated pesticides (such as DDT, toxaphene, chlordane and hexachlorocyclohexane) has been restricted or eliminated, persistent soil residues may continue to volatilize and be redistributed by the atmosphere [16]. Also, many of these same pesticides continue to be used in the lower tropical and semi-tropical latitudes.

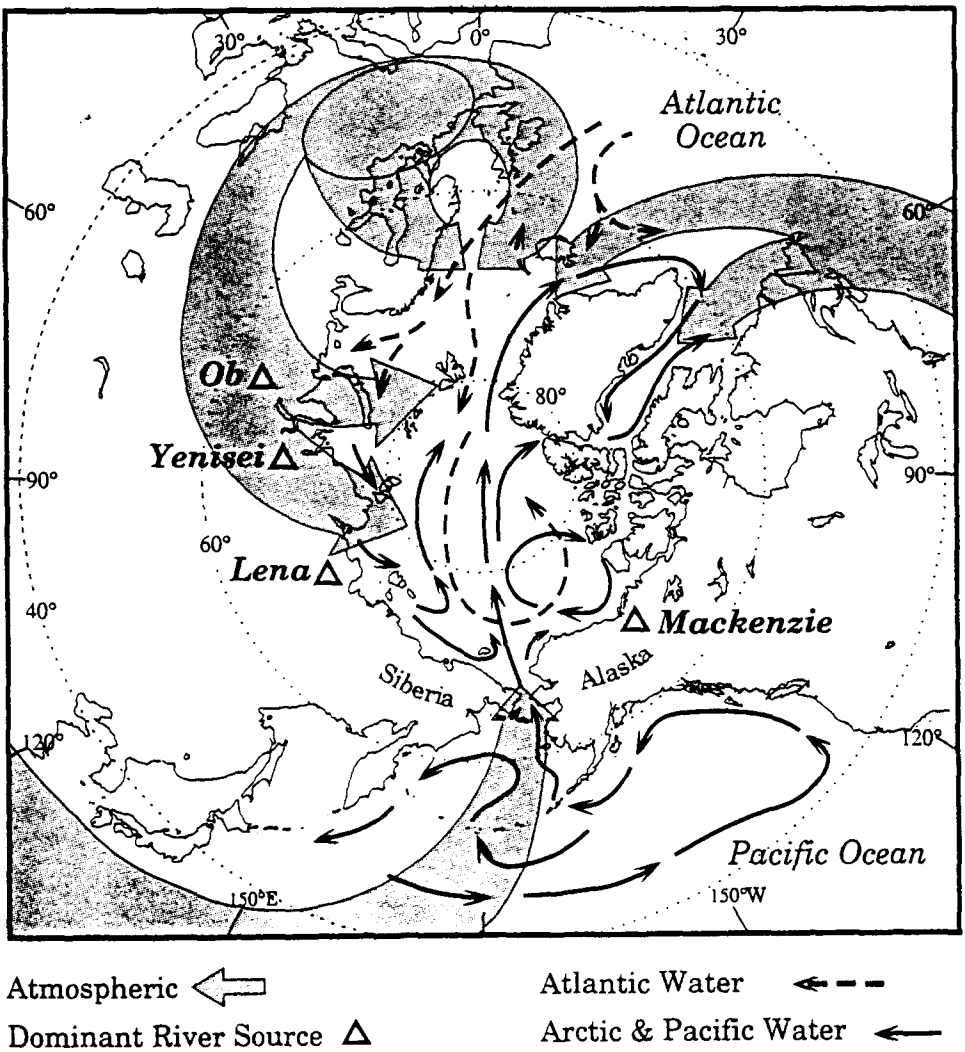

Fig. 1. Generalized Arctic transport pathways (see Refs 2–10).

THE ALASKA MARINE MAMMAL TISSUE ARCHIVAL PROJECT (AMMTAP)

Canada has routinely banked biological specimens as part of its environmental monitoring programs for many years. Similar efforts are underway in other polar nations (e.g. Finland, Norway, Sweden). The Alaska Marine Mammal Tissue Archival Project (AMMTAP) was probably the earliest organized effort to develop an environmental specimen bank specifically designed for long-term archival under cryogenic conditions of biological

specimens from the US Arctic. The project has been identified as part of the US agencies' contribution to the international Arctic Monitoring and Assessment Program [17].

The AMMTAP began in 1987 as a cooperative effort between the Arctic Environmental Assessment Center of the National Oceanic and Atmospheric Administration (NOAA) and the National Institute of Standards and Technology (NIST) to establish and conduct a program of collecting tissues from Alaska marine mammals and storing them under conditions which allow future analyses for substances indicative of contamination from offshore petroleum and mining activities. The project is funded by the Minerals Management Service (MMS), US Department of the Interior. It was believed that such a collection of samples could be used to help establish a baseline against which future impacts associated with the development of Alaska's coastal areas could be evaluated. It was also realized that such a resource could provide samples for addressing questions regarding potential environmental problems outside of the petroleum and mining industries, such as the long-distance transport of persistent contaminants from lower latitudes.

TOXICANTS IN MARINE MAMMALS

That persistent toxic substances can occur in marine mammals in high concentrations is a phenomenon that is often reported in the literature. These substances include both naturally occurring toxicants, such as the heavy metals, as well as anthropogenic substances, such as PCBs and chlorinated hydrocarbon pesticides. The relationship between high contaminant levels in body tissues and detrimental effects to these animals has been demonstrated in a few cases. For example, lowered reproductive success in pinnipeds (seals and sea lions) has been reported in regions of high pollutant loads: California sea lions on the west coast of the United States [18–20], ringed seals in the Baltic Sea [21–23] and harbor seals in the Wadden Sea [24–27].

The tendency of marine mammals to bioaccumulate contaminants can be explained by several factors including: relative position in the food web, tendency to accumulate large energy reserves in the form of body fat, relatively long life span and relative ability to metabolize and excrete toxic substances.

Relative position in the food web

Top level carnivores feed on resources already high in accumulated contaminants. The organochlorines and at least some of the organometals (such as methyl mercury) tend to biomagnify. Since most of the pinnipeds and many of the cetaceans feed at the upper level of the food web, one would ex-

pect persistent contaminants to concentrate in their tissues. Comparing contaminant levels generally found in baleen whales (plankton feeders) with toothed whales (fish feeders) tends to bear this out [28–31].

Tendency to accumulate large energy reserves in the form of body fat

Many persistent contaminants, such as organochlorine compounds, are lipophilic and tend to accumulate in body fat. As part of their thermoregulation mechanism and as a means to store energy reserves, the great majority of marine mammals develop and maintain thick layers of surface body fat (blubber). Such fat storage provides a large reservoir for the accumulation of lipophilic compounds, such as organochlorines.

Relatively long life span

Although all animals have metabolic mechanisms for breaking down and excreting persistent toxic substances, generally speaking, the longer lived the organism, the higher the accumulated contaminant load. The ability to metabolically regulate contaminants varies within a species according to age, sex, seasonal metabolism and general health of the individual animal.

Relative ability (or inability) to metabolize and excrete toxic substances

The tissue concentration level of a contaminant depends to some extent on the ability of that organism to metabolize the compound into products that can be excreted or stored in relatively innocuous forms. The ability to metabolize and excrete specific contaminants differs from species to species and not necessarily along taxonomic lines. For example, the apparent greater capacity to bioaccumulate PCBs in fish-eating birds and mammals (small cetaceans, seals, mink) as compared to other birds and mammals might be explained by lower activity of the hepatic microsomal monooxygenase system in these animals [32,33].

THE IMPORTANCE OF MARINE MAMMALS IN ALASKA

Alaska has more than half of the United States coastal area. This coastal zone and associated continental shelf extends over 20° of latitude and encompasses marine habitats ranging from those characteristic of the temperate North Pacific to those of the Arctic polar region. Within this large marine realm 34 species of marine mammals occur, including 21 species of cetaceans (whales, dolphins and porpoises), 10 species of pinnipeds (seals, sea lions and walrus), as well as sea otters, polar bears and Arctic fox.

Marine mammals are very important to Alaska. Alaska's coastal aboriginal inhabitants continue to rely upon the hunting of marine mammals as an important source of food and other raw materials. Although these subsistence patterns established in prehistoric times have been modified by modern conveniences and a cash-based economy, subsistence still plays the major role in the local economies of many remote coastal villages. Even in those less remote villages in which cash economy plays a major role, subsistence hunting, particularly of marine mammals, continues to dominate the cultural definition of the Alaska native.

Both Alaska native and non-native coastal villagers eat large quantities of foods from wildlife near the top of the food web. Considering the previous discussions concerning environmental contaminants in the Arctic derived from lower-latitude sources and the factors controlling bioaccumulation of contaminants, one would suspect that Arctic residents run the risk of being exposed to relatively high levels of environmental toxicants. Alaska Arctic residents are aware of this risk and, stimulated by recent information from the popular press on heavy metals and pesticides in marine mammals from eastern Canada [34] are becoming increasingly apprehensive as to the quality of their food and the health of the animals.

PHILOSOPHY AND APPROACH OF THE AMMTAP

Initially, four key elements were identified to be of major importance to the design of the project:

- The history of each sample has to be carefully documented
- In order to be comparable, each sample of the same tissue type has to be collected in the same manner using basically the same techniques
- Each sample has to be as fresh as possible
- Each sample has to be quick-frozen as soon as possible and maintained at temperatures low enough to insure minimum degradation during storage

As is commonly the case elsewhere, collections of marine mammal tissues exist in the freezers of many researchers and institutions in Alaska and other states. Most of these samples were collected incidental to various research projects with the idea that someone might have need for them later. These collections are usually kept until freezer space is needed for something more pertinent to the researcher, at which time the samples are discarded.

Gathering such collections and using them to establish the proposed Archive was considered. However, it was quickly decided that such collections would not be suitable. In most cases, little if any information was available on how the samples were collected and handled in the field, how soon after the death of the animal the samples were collected, how much time elapsed

between sampling and freezing of the samples and at what temperatures the samples were really kept during storage. Such missing information is necessary to insure the integrity of the samples which is particularly important considering the high cost of analysis for many of the toxic substances of interest.

It was apparent that the development of a high quality sample collection required that samples be obtained using standardized rigorous sampling protocols developed specifically for the project to insure that the individual samples from the same tissue types are comparable and that the history of the sample from time of collection until analysis is documented. A key factor in NIST's involvement in the project was the Institute's extensive experience in the development of detailed and rigorous tissue sampling procedures and its sophisticated facility for the cryogenic storage of these samples. The development of the NIST facility, the National Biomonitoring Specimen Bank (NBSB), has been described previously [35,36].

It was also decided that samples would only be taken from healthy animals immediately following death, not stranded animals, most of which would be in various stages of decomposition. This decision initially presented a problem, since the justification for intentionally killing marine mammals to obtain such samples might be considered dubious. Therefore, it was decided to arrange for the sampling of animals taken by other researchers possessing permits for taking marine mammals as part of their own research programs and to sample animals taken for food by Alaska native hunters. The latter approach has proven to be of greatest value. So far all samples taken by the AMMTAP have been from animals taken by native subsistence hunters.

TISSUE TYPES SELECTED FOR SAMPLING

The tissues originally selected for routine sampling were blubber, liver, kidney and muscle. The selection of these four tissues/organs was based on the following criteria [37]:

- The tissue is accessible to sampling techniques
- The tissue has the potential for concentrating both inorganic and organic substances
- The sample is conducive to precise anatomical description
- The tissue will provide a homogeneous sample
- A minimum of two 150 g samples can be obtained from the tissue

All four of these tissues/organs also represent items consumed as food by the Alaska natives, the importance of each varying with species, village traditions and individual tastes.

The principle tissues chosen for sampling were blubber (the layer of fat lying between the skin and muscle) and liver. Blubber, due to its high lipid content, concentrates organic toxicants to relatively high levels. The liver is a major detoxification site for xenobiotics and is suitable for measuring all known environmental toxicants plus biotoxins. The liver generally has sufficient lipid content that it is suitable as an accumulator of organic as well as inorganic substances and may also have a higher proportion of metabolites than other tissues. Because of the tendency for several of the toxic metals (particularly Cd) to concentrate to relatively high levels in the kidney, this organ was also selected to be routinely collected.

After the first year of sampling, muscle was deleted from the list of tissues routinely archived by the project. This decision was due to the relatively low levels of toxicants usually associated with muscle tissue as compared to the other three tissue types, the difficulty in obtaining a uniform sample uncontaminated by intermuscular fat and connective tissue, as well as the difficulty in arriving at homogeneous analytical aliquots during cryogenic homogenization of the samples.

In addition to the three tissues collected for cryogenic storage, other samples that can aid in interpreting the results of chemical analyses of these principal tissues are collected from the animals. These additional samples include liver and kidney subsamples in buffered formalin for histology, teeth for age determination, bile for PAH metabolite screening (periodically), stomach contents for food identification (periodically) and, more recently, subsamples of liver and muscle for genetics studies (University of Alaska Frozen Tissue Collection) and blood serum for pathology studies (Alaska Department of Fish and Game's wildlife serum library).

PROTOCOLS

The details of the protocols used by the AMMTAP for sampling and archival have been presented and discussed previously [37,38]. The intent of these protocols is to provide a consistent and carefully documented procedure for sampling, to develop and maintain a detailed record of sample history, to insure that the samples are kept in the best conditions for long-term storage without loss of original sample integrity and to use procedures and equipment that minimize the chance of introducing artifacts to the sample that might bias future chemical analytical results. For example, to avoid contamination from trace elements commonly found in conventional dissection instruments (e.g. Ni, Cr and Fe), samples are excised using titanium-bladed knives. Excised organs and samples are stored in Teflon bags. During sample preparation, contact with the specimen is limited to clean, dust-free Teflon surfaces and samples are frozen and permanently stored in Teflon jars. As

soon as possible after collection and preparation, samples are frozen in liquid nitrogen (LN_2), maintained in LN_2 vapor during shipment to the archive and are stored in the NBSB LN_2 freezers at NIST at LN_2-vapor temperature (−150°C) until analyzed. These conditions appear to be the best for long-term storage of the samples. Previous assessment of the long-term stability of environmental specimens in the NBSB indicate no change following 7 years of storage in LN_2 vapor [36].

Because of the nature of the principal source of specimens (Alaska native subsistence hunts), the sampling protocols developed by the AMMTAP required careful consideration of the hunting procedures used and logistical problems in transporting sampling equipment and materials to remote locations having highly variable and often unpredictable weather and environmental conditions. With the intent of not sacrificing the rigorous nature of the sampling procedures, steps had to be taken to adapt these procedures to standard hunting practices in order to insure cooperation of the hunters. In many cases where it appeared such adaptations would lead to questionable sample integrity, sampling opportunities were passed up.

Two 150-g subsamples, A and B, are collected for each tissue type from each animal sampled. Subsample A is intended to be maintained in the specimen bank for future retrospective analysis. Subsample B, although archived under the same conditions as subsample A, is available for homogenization and subsampling into aliquots for more immediate analysis for quality assurance or other purposes if required.

The sampling data form used by the AMMTAP is designed to provide a record of the history of each sample. Information entered into the form records the manner in which the animal died, environmental conditions at the time of sample collection, additional samples taken from the animal, measurement data for each animal, any modifications of the standard protocol that had to be followed because of unusual conditions and a time-track of sample handling (e.g. time of death, time of sampling, time of sample preparation, time of sample freezing, time shipped from collection site and time received at the specimen bank).

Information for each sample (including field collection data) is maintained at the NBSB in both hard copy and in a computer data base as part of the sample documentation. An NBSB number is assigned to each sample and the samples are placed in LN_2 freezer storage until requested for analysis. For security purposes, subsamples A and B are stored in different LN_2 freezers.

As part of the specimen banking procedures, aliquots of 10–20% of the specimens (taken from subsamples B) are analyzed to determine the concentrations of selected organic and inorganic constituents. These analyses provide data for evaluating the stability of the specimens during long-term

storage. In addition, the data provide some real-time measure of contaminant concentrations for monitoring purposes, provide a baseline for comparison with results from samples collected in the future to monitor long-term trends in pollution and provide a comparison with data obtained by other laboratories on subsamples from the AMMTAP, or similar samples collected at the same time from the same sites (i.e. quality assurance).

Samples to be analyzed are homogenized using a cryogenic grinding procedure designed to minimize sample contamination and reduce the likelihood of changes in sample composition due to thawing and refreezing [39]. The grinding procedure, which uses Teflon disk mills, is capable of homogenizing 150-g sample aliquots to provide homogeneous frozen samples with greater than 90% of the particles less than 0.46 mm in diameter and with subsampling errors due to inhomogeneity estimated at less than 2% [36].

Although initial analytical work was conducted on all tissue-types, analyses of specimens since 1990 have focused on analyzing only liver for trace elements and only blubber for organic constituents. PCBs and chlorinated pesticides have been the focus of the organic analyses since they readily accumulate in the fatty tissues and are generally measured in monitoring programs. Inorganic analysis has routinely involved the determination of 36 trace elements. Specific constituents routinely analyzed by the specimen bank are presented in Table 1. Detailed descriptions of the analytical methods and the results of analyses of tissues from 12 animals are presented elsewhere [41,42].

RESULTS OF THE AMMTAP

The first year of the AMMTAP (1987) was devoted to the design and testing of field sampling protocols. These initial tests were conducted during the subsistence harvest of the northern fur seals on St. Paul Island, Bering Sea, in July, 1987. The protocols were evaluated as to their practicality and suitability for obtaining samples of the four tissue types (liver, kidney, blubber and muscle) which were uncontaminated during collection and handling. Based on the results from the St. Paul work, the protocols were revised and published [37]. Subsequent field sampling of other species resulted in a second protocol revision in 1991 [38].

A total of 196 tissue specimens have been collected and archived by the AMMTAP through 1991. These specimens were collected from 65 individual animals representing seven species (ringed seal, *Phoca hispida*; spotted seal, *P. largha*; harbor seal, *P. vitulina*; bearded seal, *Erignathus barbatus*; northern fur seal, *Callorhinus ursinus*; Steller or northern sea lion, *Eumatopias*

TABLE 1

Organic and inorganic constituents routinely measured in the AMMTAP. Analytical methods are: gas chromatography with electron capture detection (GC-ECD), instrumental neutron activation analysis (INAA), differential pulse anodic stripping voltammetry (DPASV) and cold vapor atomic absorption spectrometry (CVAAS)

Organic constituents (GC-ECD)

PCB congeners
IUPAC-8, 18/15, 28, 44, 52, 66/95, 101/90, 105, 118, 138/163/164, 153, 170/190, 180, 187/159/182, 195
2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 4,4′-DDD, 2,4′-DDT, 4,4′-DDT
Hexachlorobenzene (HCB)
γ-Hexachlorocyclohexane (gamma-HCH)
Dieldrin
Heptachlor epoxide
cis-Chlordane
trans-Nonachlor

Inorganic constituents

Na (INAA)	Ni (DPASV)	Cs (INAA)
Mg (INAA)	Cu (INAA, DPASV)	La (INAA)
Al (INAA)	Zn (INAA, DPASV)	Ce (INAA)
Cl (INAA)	As (INAA)	Sm (INAA)
K (INAA)	Se (INAA)	Eu (INAA)
Ca (INAA)	Rb (INAA)	Tb (INAA)
Sc (INAA)	Sr (INAA)	Hf (INAA)
V (INAA)	Mo (INAA)	Ta (INAA)
Cr (INAA)	Ag (INAA)	Au (INAA)
Mn (INAA)	Cd (INAA, DPASV)	Hg (CVAAS)
Fe (INAA)	Sb (INAA)	Pb (DPASV)
Co (INAA, DPASV)	I (INAA)	U (INAA)

jubatus; and beluga or northern white whale, *Delphinapterus leucas*) from the following locations:

Northern fur seal	St. Paul I., Bering Sea (1987, 1990)
Ringed seal	Barrow, Chukchi Sea (1988, 1991)
	Nome, Norton Sound (1989, 1991)
Bearded seal	Barrow, Chukchi Sea (1989)
	Nome, Norton Sound (1989)
Spotted seal	Nome, Norton Sound (1991)
Harbor seal	Prince William Sound (1990)
Northern sea lion	Cook Inlet (1990)
Beluga whale	Point Hope, Chukchi Sea (1989)
	Point Lay, Chukchi Sea (1990)

Half of the specimens were collected from pinnipeds and cetaceans in the Arctic Ocean and the other half were collected from pinnipeds in the Bering Sea and Northern Gulf of Alaska (Fig. 2). The specimen bank inventory provided in Tables 2 and 3 also indicates those specimens that have been homogenized and analyzed by the specimen bank. Results of these analyses are presented elsewhere [40] and are summarized and discussed in two separate papers in this volume [41,42].

EVALUATION OF THE ORIGINAL CRITERIA USED TO SELECT SPECIES FOR SAMPLING

The original criteria used to select the species to be sampled have been presented and discussed by Becker et al. (1988) [37]:

- Geographic range of the species
- Position in the food web
- Human subsistence use

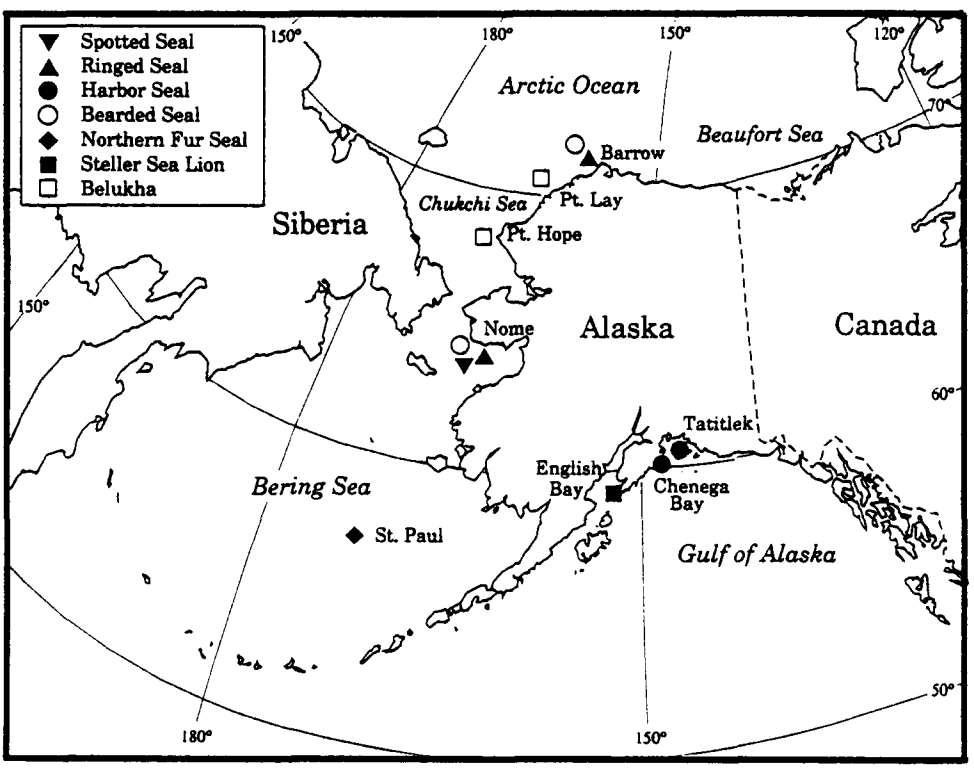

Fig. 2. Marine mammals sampled by the Alaska Marine Mammal Tissue Archival Project.

TABLE 2

Marine mammals sampled in the Arctic Ocean (×, specimens archived; ■, subsample B homogenized and divided into aliquots for analyses; □, aliquots of subsample B have been analyzed)

Species	Sex	Individual ID	Location	Date	Tissue			
					L	K	B	M
Ringed seal	M	692-RGSL-001	Barrow	7/88	×		×	×
Ringed seal	F	692-RGSL-002	Barrow	7/88	×		×	×
Ringed seal	M	692-RGSL-003	Barrow	7/88	×		×	×
Ringed seal	M	692-RGSL-004	Barrow	7/88	□	□	□	
Ringed seal	F	692-RGSL-005	Barrow	7/88	×		×	×
Ringed seal	F	692-RGSL-006	Barrow	7/88	×		×	×
Ringed seal	M	692-RGSL-007	Barrow	7/88	×		×	×
Ringed seal	M	692-RGSL-008	Barrow	7/88	□	□	□	
Ringed seal	M	692-RGSL-009	Barrow	7/88	×		×	×
Ringed seal	F	692-RGSL-010	Barrow	7/88	×		×	×
Ringed seal	M	692-RGSL-019	Barrow	7/91	×		×	×
Ringed seal	M	692-RGSL-020	Barrow	7/91	×		×	×
Ringed seal	M	692-RGSL-021	Barrow	7/91	×		×	×
Ringed seal	M	692-RGSL-022	Barrow	7/91	×		×	×
Ringed seal	F	692-RGSL-023	Barrow	7/91	×		×	×
Ringed seal	M	692-RGSL-024	Barrow	7/91	×		×	×
Ringed seal	F	692-RGSL-025	Barrow	7/91	×		×	×
Ringed seal	M	692-RGSL-026	Barrow	7/91	×		×	×
Ringed seal	M	692-RGSL-027	Barrow	7/91	×		×	×
Ringed seal	F	692-RGSL-028	Barrow	7/91	×		×	×
Bearded seal	M	692-BDSL-002	Barrow	7/89	×		×	×
Bearded seal	M	692-BDSL-003	Barrow	7/89	×		×	
Belukha whale	F	692-BLKA-001	Pt. Hope	5/89	□	×	□	
Belukha whale	F	692-BLKA-002	Pt. Hope	5/89	□	×	□	
Belukha whale	F	692-BLKA-003	Pt. Hope	5/89	□	×		
Belukha whale	M	692-BLKA-004	Pt. Hope	5/89	□			
Belukha whale	F	692-BLKA-005	Pt. Lay	7/90	×	×		■
Belukha whale	M	692-BLKA-006	Pt. Lay	7/90	×	×		■
Belukha whale	F	692-BLKA-007	Pt. Lay	7/90	□	×	□	
Belukha whale	M	692-BLKA-008	Pt. Lay	7/90	×	×		■
Belukha whale	M	692-BLKA-009	Pt. Lay	7/90	×	×		■
Belukha whale	M	692-BLKA-010	Pt. Lay	7/90	×	×		■
Belukha whale	M	692-BLKA-011	Pt. Lay	7/90	×	×		■
Belukha whale	F	692-BLKA-012	Pt. Lay	7/90	□	×	□	
Belukha whale	M	692-BLKA-013	Pt. Lay	7/90	×	×		■
Belukha whale	F	692-BLKA-014	Pt. Lay	7/90	×	×		■

TABLE 3

Marine mammals sampled in the Bering Sea and Gulf of Alaska. (PWS, Prince William Sound; ×, specimens archived; ■, subsample B homogenized and divided into aliquots for analyses; □, aliquots of subsample B have been analyzed)

Species	Sex	Individual ID	Location	Date	Tissue			
					L	K	B	M
Ringed seal	M	692-RGSL-011	Nome	5/89	□	×	□	
Ringed seal	F	692-RGSL-012	Nome	5/89	×	×	×	
Ringed seal	M	692-RGSL-013	Nome	5/89	□	×	□	
Ringed seal	M	692-RGSL-014	Nome	5/89	×	×	×	
Ringed seal	F	692-RGSL-015	Nome	5/89	×	×	×	
Ringed seal	M	692-RGSL-016	Nome	5/91	×	×	×	
Ringed seal	F	692-RGSL-017	Nome	5/91	×	×	×	
Ringed seal	F	692-RGSL-018	Nome	5/91	×	×	×	
Spotted seal	F	692-SPSL-001	Nome	5/91	×	×	×	
Bearded seal	M	692-BDSL-001	Nome	5/89	×	×	×	
N. fur seal	M	692-FRSL-001	St. Paul	7/87	×	×	×	×
N. fur seal	M	692-FRSL-002	St. Paul	7/87	×	×	×	×
N. fur seal	M	692-FRSL-003	St. Paul	7/87	×	×	×	×
N. fur seal	M	692-FRSL-004	St. Paul	7/87	□	□	□	□
N. fur seal	M	692-FRSL-005	St. Paul	7/87	□	□	□	□
N. fur seal	M	692-FRSL-006	St. Paul	7/90	×	×	×	
N. fur seal	M	692-FRSL-007	St. Paul	7/90	□	×	×	
N. fur seal	M	692-FRSL-008	St. Paul	7/90	×	×	×	
N. fur seal	M	692-FRSL-009	St. Paul	7/90	×	×	×	
N. fur seal	M	692-FRSL-010	St. Paul	7/90	□	×	×	
N. fur seal	M	692-FRSL-011	St. Paul	7/90	□	×	×	
N. fur seal	M	692-FRSL-012	St. Paul	7/90	×	×	×	
N. fur seal	M	692-FRSL-013	St. Paul	7/90	×	×	×	
N. fur seal	M	692-FRSL-014	St. Paul	7/90	×	×	×	
N. fur seal	M	692-FRSL-015	St. Paul	7/90	×	×	×	
Harbor seal	M	692-HBSL-001	PWS	3/90	×	×	×	
Harbor seal	F	692-HBSL-002	PWS	4/90	×	×	×	
Harbor seal	M	692-HBSL-003	PWS	4/90	×	×	×	
S. sea lion	F	692-STSL-001	Cook Inlet	3/90	×	×	×	

- Availability of baseline biological information
- Practicality of collecting fresh samples following protocols

Table 4 presents a matrix showing the animals sampled by the project as they relate to these criteria. Half of the animals sampled have broad geographical ranges, being circumpolar in distribution (ringed seal, bearded seal and beluga whale). Most are pelagic fish feeders; only one benthic feeder has been sampled (bearded seal) and the ringed seals represent animals that, depending on age, feed from the mid-level of the food web on pelagic crustaceans (shrimp and amphipods) to the upper-level finfish. All species except one (Steller sea lion) are fairly important subsistence species, and all have fairly good baseline biological data bases. From the standpoint of ease in collecting fresh samples on a routine basis using the protocols, the highest marks go to ringed seal, northern fur seal and beluga whale.

Of the animals that have been sampled for the AMMTAP, the best candidates for circumpolar comparisons and monitoring for environmental contaminants are probably the ringed seal and the beluga whale. Both are widely

TABLE 4

Species selection criteria as related to those species sampled by the AMMTAP and those species which are candidates for future sampling

Species	Geographic Range	Food Web Position	Sub-sistence Value	Baseline Bio-Data	Sampling Practicality
Species sampled					
Ringed Seal	Circumpolar	Pelagic Fish Crustaceans	High	Very Good	Excellent
Harbor Seal	World-wide	Pelagic fish	Moderate	Very Good	Difficult
Spotted Seal	W.-Arctic Bering Sea	Pelagic fish	High	Limited	Moderate
Bearded Seal	Circumpolar	Benthos	High	Limited	Difficult
Steller Sea Lion	North Pacific	Pelagic fish	Low	Moderate	Difficult
Northern Fur Seal	North Pacific Bering Sea	Pelagic fish; squid	Moderate	Very Good	Excellent
Beluga Whale	Circumpolar	Pelagic fish	High	Moderate	Excellent
Candidates for future sampling					
Walrus	Broadly Polar	Benthos	High	Moderate	Difficult
Bowhead Whale	Broadly Polar	Pelagic plankton	High	Moderate	Moderate
Polar Bear	Circumpolar	Top Predator	High	Moderate	Moderate

distributed in the Arctic (Figs 3 and 4). The ringed seal has been studied extensively and a relatively good contaminants data base exists for this species on a world-wide basis. For reference purposes, populations of this species from the Baltic Sea have been reported to have high enough levels of PCBs and other organochlorine compounds to contribute to a lowered reproductive capacity [21–23].

Although the data base for the beluga whale are not as extensive as that for the ringed seal, this animal has a high potential for concentrating contaminants in its tissues (it feeds at the top of the marine food web) and it has recently been the object of environmental contaminants research across the

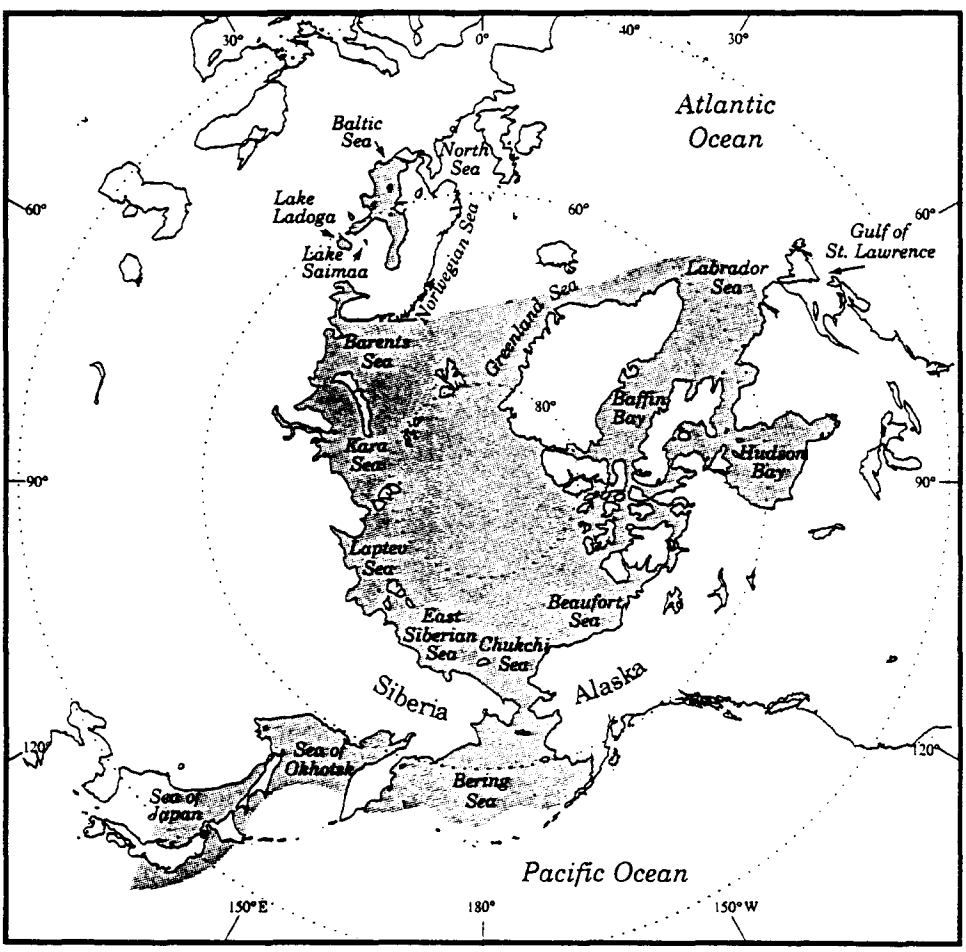

Fig. 3. Circumpolar distribution of the ringed seal (*Phoca hispida*).

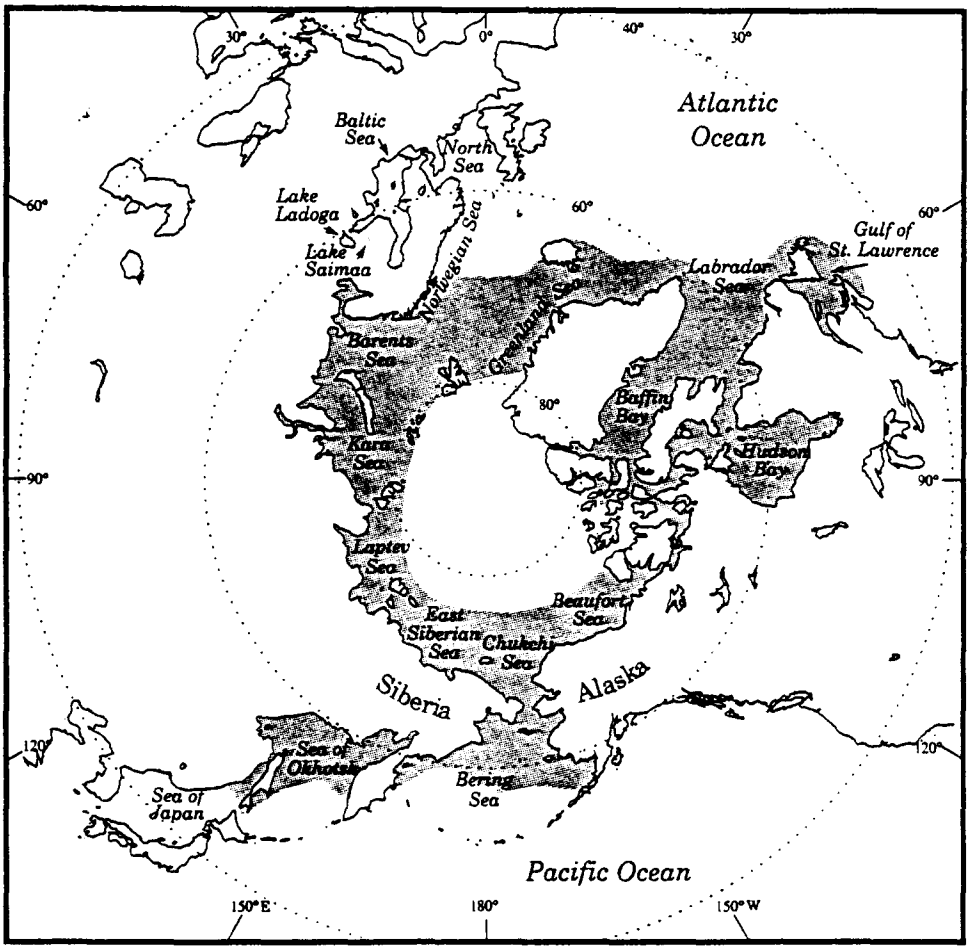

Fig. 4. Circumpolar distribution of the beluga or northern white whale (*Delphinapterus leucas*).

Arctic from Greenland through Canada to the Bering Strait. Belugas from the Gulf of St. Lawrence have been reported to have relatively high levels of heavy metals [43] and organochlorine compounds such as PCBs [44]. The sources of these contaminants are probably the agricultural and industrial areas located in the drainage basins emptying into the Gulf plus atmospheric transport from other regions [44]. This population of belugas could provide a reference data base for comparing with other populations of this species found in remote areas of the Arctic and which are not exposed to local pollution sources.

Candidates for future sampling in the AMMTAP are the walrus, bowhead

whale and polar bear. Both the walrus and bowhead are very important subsistence species. The walrus is only one of two benthic feeding species targeted by the AMMTAP, the other being the bearded seal. Based on its trophic position, one would not expect the bowhead to concentrate environmental contaminants to the same level as the beluga whale. However, from a human subsistence value standpoint it is probably the most significant marine mammal for most Alaska native coastal villages north of the Bering Strait.

Of those candidates for future sampling by the AMMTAP, the polar bear may be one of the most valuable for environmental monitoring. This species is 'the' top predator of the Arctic. It is circumpolar in distribution, its range roughly coinciding with that of the ringed seal and the beluga whale. The ringed seal provides its most important food base in the form of skin and blubber. The polar bear has also been known to prey on walrus, beluga and gray whales trapped in the Arctic ice and it is not opposed to preying on humans if that is the only food source available. Studies of organochlorines in polar bear as related to concentrations in ringed seals and its prey, Arctic cod, have been conducted in the Canadian Arctic and have produced some interesting results, particularly as related to the metabolism of PCB congeners through the food chain [45,46].

CONSIDERATIONS FOR REDUCING DATA VARIABILITY AS RELATED TO SPECIMEN BANKING

Reijnders (1986) and Aguilar (1987) have discussed the factors producing high variability in contaminant levels measured in marine mammals (Table 5) [47,48]. Collection, storage and analytical conditions should be exactly the same for all samples in order for comparisons between samples to be valid. Samples preserved under different conditions are not directly comparable unless the possibility of differential pollutant derivation or loss can be reasonably excluded [48]. The collection protocols used by the AMMTAP are designed to control such variation through appropriate design of sampling, preservation and analytical techniques.

Besides those factors which are controllable by standardized collection and analytical procedures, other variability factors are determined by the nature of the animal itself and how it is exposed to the contaminant (Table 5). Although perhaps not strictly controllable during sampling, knowledge of these variability factors can be used to decide which comparisons can be made and the interpretations that can be derived from these comparisons. In addition, if appropriate biological data are available for each animal when samples are selected from the specimen bank for analysis, many of these factors are controllable. For example, Aguilar points out that, ideally, any com-

TABLE 5

Factors producing high variability in contaminant levels measured in marine mammals

Determined by collection, treatment and analytical protocols
- Physiological compartment selected for analysis
- Tissue sampling procedures
- Tissue preservation procedures
- Analytical procedures
- Specific compounds selected for expressing contaminant burden (isomers, congeners, metabolites, etc.)
- Units selected for expressing concentrations (wet wt, dry wt, lipid fraction)

Determined by the nature of the animal and how it is exposed to contaminant
- Species specific differences
- Age of the animal
- Sex of the animal
- Position of the animal in the food web (commonly varies with age and sex)
- Physiological state of the animal:
  - Reproductive state
  - Feeding state
  - Seasonal changes in stored energy reserves
  - General health of the animal
- Variation in exposure to the contaminant
- Differential metabolism and excretion of the contaminant

parison of pollutant concentrations between different marine mammal populations should use only individuals of the same age, sex, reproductive category and fattening state [48]. He also suggests that sampling within a given size range of only one sex (preferably males because of the usual higher tissue loads) on a limited time period would help to overcome many of the variability problems.

Within the AMMTAP, the ability to direct the sampling such that the above restrictions apply is difficult. The Project relies on samples collected in cooperation with local native subsistence hunters and ongoing research and management programs of other agencies; therefore, such control is not always feasible. However, for some pinniped species selection of animals based on sex and age is possible. For example, northern fur seal samples obtained during the subsistence harvest come from 2- to 3-year-old males, since these are the only animals taken during the harvest. In addition, one can select specific sex and age ranges for analysis if the number of banked specimens for each species is large enough and the individual animal data that are recorded during collection are maintained.

COORDINATION WITH OTHER PROGRAMS AND ORGANIZATIONS

The AMMTAP is somewhat unique in that the nature of the resource being addressed and the principal source of the samples require extensive coordination with many different organizations inside and outside Alaska. The AMMTAP has placed particular emphasis on establishing and maintaining a close working relationship with native organizations and international organizations in which Alaska natives play a prominent role. To date, this has involved periodic and regular meetings with 14 different Alaska native organizations.

In addition, AMMTAP is collaborating and coordinating its work with several major research and marine mammal management programs both inside and outside Alaska (Table 6). This has included mutual field work during sampling (Beluga Harvest Survey; Global Baseline Pollution Studies), collaboration on protocol design (National Marine Mammal Tissue Bank), collection of supplemental samples for genetics research (Alaska Frozen Tissue Collection), pathology (Alaska Department of Fish and Game's wildlife serum library) and studies related to specific pollution events (Prince William Sound Subsistence Foods Monitoring Program in response to the EXXON VALDEZ oil spill), as well as collaboration on chemical analyses (Department of Fisheries and Oceans Canada, Nuclear Research Center, Jülich and the University of Ulm, Germany).

OTHER BIOLOGICAL SPECIMEN BANKING ACTIVITIES IN ALASKA

Besides the AMMTAP, other Alaska cryogenic biological specimen banks designed for long-term storage for retrospective analysis are the Alaska Department of Fish and Game's (ADF&G) wildlife serum library and the emerging Alaska Frozen Tissue Collection of the University of Alaska Museum (Table 7). While the AMMTAP is designed to answer questions regarding environmental contaminant concentrations in marine mammals, ADF&G's wildlife serum library was established to provide blood sera for addressing questions regarding viral infections and other wildlife diseases and the Alaska Frozen Tissue Collection is specifically designed to provide a storehouse of materials for genetics and systematics research on mammals. The AMMTAP is coordinating its activities with these other specimen banks in order to maintain a cross reference of specimens and associated information.

Although most of the blood sera in ADF&G's collection are from terrestrial species, serologic evidence indicating exposure of Alaska phocid seals to the seal herpesvirus (SeHV), a virus prevalent in phocid seals of the North Atlantic and North Sea, has stimulated sampling of Alaska pinnipeds [49].

TABLE 6

Programs with which the AMMTAP is collaborating or coordinating its work

Program	Organization	Activity
National Marine Mammal Tissue Bank	National Marine Fisheries Service, Silver Spring, Maryland, USA	Protocol design
Circumpolar Distribution of Oganochlorine Compounds in Beluga Whales	Department of Fisheries and Oceans Canada, Winipeg, Manitoba, Canada	Chemical analysis
Beluga Harvest Survey	North Slope Borough Department of Wildlife Management, Barrow, Alaska, USA	Cooperative field work
Alaska Frozen Tissue Collection	University of Alaska Museum, Fairbanks, Alaska, USA	Supplemental samples
Wildlife Serum Archive	Alaska Department of Fish & Game, Fairbanks, Alaska, USA	Supplemental samples
Prince William Sound Subsistence Foods Monitoring Program	Alaska Department of Fish & Game, Anchorage, Alaska, USA	Supplemental samples
Environmental Specimen Bank Program	Nuclear Research Center, Jülich, Germany	Chemical analysis
Global Baseline Pollution Studies	Dept. of Analytical Chemistry, University of Ulm, Ulm, Germany	Cooperative field work, chemical analysis

Surveys of Alaska phocids conducted during the 1980s revealed no evidence of exposure to phocid distemper virus which killed so many seals during that decade in Northern Europe. Routine banking of pinniped serum will provide an important resource for the monitoring of viral and bacterial pathogens in the Alaska populations.

The Alaska Frozen Tissue Collection is a new program based on recommendations for a national plan on management of frozen tissue collections [50]. With the advent and widespread use of new genetic technologies, cryogenically preserved tissue specimens can a play an important role in ad-

TABLE 7

Alaska biological specimen banks established for retrospective analysis

Program, Specimen Bank	Specimens	Principal Use	Contact
Alaska Marine Mammal Tissue Archival Project National Biomonitoring Specimen Bank	Liver, kidney, and blubber from marine mammals	Environmental contaminants analyses	Paul R. Becker[a] or Stephen A. Wise[b]
Alaska Frozen Tissue Collection, University of Alaska Museum	Heart, liver, kidney and skeletal muscle from mammals	Genetics research	Joseph A. Cook[c]
Alaska Department of Fish and Game's wildlife serum library	Blood serum from birds and mammals	Infectious disease survey	Randall L. Zarnke[d]

[a]Paul R. Becker, NMFS, 4335 East Wing Hwy, Silver Spring, MD MD 20910. Phone (301) 713-2319.

[b]Stephen A. Wise, NIST, Chemical Science and Technology Laboratory, Gaithersburg, MD 20899. Phone (301) 975–3112.

[c]Joseph A. Cook, University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775. Phone (907) 474–7505.

[d]Randall L. Zarnke, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, AK 99701. Phone (907) 456–5156.

dressing some of the critical biological issues of the day, such as the increasing rate of loss of genetic and biological diversity (J.A. Cook, University of Alaska Museum, Fairbanks, personal communication, 1991). The Alaska Frozen Tissue Collection is being designed principally as a genetic databank for future investigators of Alaska mammals. It will provide a reference library of Alaska's vertebrate species for studies of population genetics, pathology, specimen identity for law enforcement and systematics. Tissues typically collected for the Alaska Frozen Tissue Collection are heart, skeletal muscle, liver and kidney. Samples are stored in 2 ml cryovials ('nunc tubes') in ultracold freezers (-70°C).

CONCLUSIONS

Biological specimen banking has the potential for becoming an important part of environmental research and monitoring in the Arctic. Since 1987, the Alaska Marine Mammal Tissue Archival Project has been collecting tissues from marine mammals in Alaska and banking them in the National

Biomonitoring Specimen Bank with the intent of establishing a representative collection for future retrospective environmental contaminant analyses as part of an effort to document long-term trends in environmental quality.

Presently, the specimen inventory is relatively small. However, the careful nature in which the samples are collected and the rigorous protocols that are followed between sampling and analysis should make this collection of particular value for future analytical work on environmental contaminants of which we at present may know very little.

The project has been and will continue to emphasize coordination and collaboration with other researchers involved in contaminant and marine mammal research in the Arctic. It will also continue to encourage the development of other biological specimen banks in the Arctic and particularly in Alaska.

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