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Delphinapterus leucus

Mitochondrial DNA sequence variation within and among North American populations

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ABSTRACT

Beluga whales are migratory over much of their range, congregating in small groups around shallow river estuaries in summer, and overwintering in large groups in areas with reliable open water. This complicates management issues because it is unclear if belugas from the common wintering ground represent one large group with exchange of individuals, or if each summer estuarine concentration should be managed as a separate stock.

To examine the genetic structuring, we analyzed variation in mitochondrial DNA (mtDNA) restriction sites among 101 beluga whales from 10 regions across North America, including Greenland. Using 11 restriction enzymes, 9 haplotypes were identified among 71 whales. The remaining 30 whales were tested with only the six restriction enzymes found to identify polymorphisms. We found a marked segregation of divergent haplotypes for both sexes between eastern and western Hudson Bay. Haplotype 1 was found in 19 out of 21 animals on the east coast, while haplotype 5 was found in 18 out of 20 animals on the west coast. Sequence divergence among the 71 belugas was estimated to be 2.03%. Haplotypes fell into two major phylogenetic groups, labelled lineage I and II. Lineage I haplotypes occurred primarily in the St. Lawrence Estuary and the eastern Hudson Bay. Lineage II haplotypes occurred primarily along the western Hudson Bay, Southern Baffin Island, western Greenland, the Canadian high arctic, and the Beaufort Sea. These findings support the hypothesis that belugas exhibit maternally directed

philopatry to summering grounds, and are consistent with the hypothesis that after deglaciation, the arctic was recolonized by at least two stocks of belugas divergent in their mtDNA, possibly representing Atlantic and Pacific stocks.

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"If you have built castles in the air,
your work need not be lost;
that is where they should be.
Now put the foundations under them."

Henry David Thoreau

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GENERAL INTRODUCTION

Beluga whales (*Delphinapterus leucas*) are hunted over much of their range. This presents both a problem and an opportunity. The problem arises from the fact that in some areas they have been over harvested and thus must be carefully managed to avoid further depletion (Reeves and Mitchell, 1989; Richard, 1991). Information on population genetic structure of beluga whales is critical in order to make wise management decisions. The opportunity arises from the hunt itself. A great deal can be learned about beluga biology from the animals that are killed (Sergeant, 1973; St. Aubin et al. 1990). This study was initiated with two goals in mind. The first was to use hunter collected samples to work out the laboratory and field logistics (such as sample preservation) of doing molecular genetic research on cetaceans. This work has been published (Helbig et al., 1989; Brown et al., 1991). The second goal was to examine the population genetic structure of beluga whales using mitochondrial DNA analysis. This is presented here as a manuscript prepared for submission for publication. The research was conducted jointly by myself and researchers in Dr. Jim Clayton's laboratory at the Freshwater Institute in Winnipeg. I analyzed 71 beluga whale samples using 11 restriction enzymes for restriction fragment length polymorphisms (RFLP's). Researchers in Dr. Clayton's lab analyzed 30 animals using 6 restriction enzymes. Using the joint data set, I have analyzed the data, performed statistical tests where appropriate, drafted the figures and tables, and written the manuscript. As the study progressed it became apparent that a better understanding of

beluga population structure would be gained by including samples from specific areas where, as it happens, belugas are not hunted. I conducted a pilot project to test the feasibility of collecting skin samples from free-ranging beluga whales using a biopsy dart. A brief summary of this project with recommendations is included in Appendix I.

Information on the population genetic structure of beluga whales is both interesting from an evolutionary perspective and very important for the management of threatened and endangered populations of belugas (Reeves and Mitchell, 1989; Richard, 1991). In order to put our results into context, I have included background information that reviews aspects of beluga natural history that may influence their population structure.

Background

The beluga whale is a medium sized all white odontocete with a circumpolar distribution. Adaptations to living in the arctic include loss of the dorsal fin, development of thick skin, and increased dive duration. Belugas can live in pack ice and travel for 2 to 3 km under the ice before surfacing for air (Kleinenberg 1964). They apparently use sound to find breathing holes (Gurevich 1980) and if necessary can break ice up to 8 cm thick with their backs or heads (Kleinenberg 1964). Even with these adaptations, ice cover plays an extremely important role in restricting the distribution of belugas on a seasonal basis as well as through geological time. This in turn is expected to affect the genetic structure of the species since isolated populations

are likely to diverge with time to become separate species, subspecies, or merely populations with varying degrees of genetic differentiation.

Genetic variation is the raw material upon which evolution can operate. While differences between individuals ultimately arise through mutation, variation between populations arises through genetic drift and selection for adaptation to local conditions (Rockwell and Barrowclough 1987). The degree to which populations diverge under these influences depends primarily upon effective population size and the extent of genetic isolation. Conversely, the degree of genetic continuity maintained across a species range depends on the level of gene flow (where gene flow is defined as the movement and incorporation of alleles among local populations) (Rockwell and Barrowclough 1987). The genetic organization of a species that results from the balance between these opposing forces is known as the "genetic population structure" (also referred to simply as "genetic structure" or "population structure"). The genetic structure is intended to reflect the effective population size, breeding system, and the degree of connectedness of local demes through gene flow. As Barrowclough (1983) states, "genetic structure is an attribute of populations that is of crucial importance for the quantitative understanding of the mechanisms and dynamics of micro evolution, including speciation".

The pattern and amount of genetic structuring of a species is influenced not only by the biological potential for gene flow (i.e.

mobility of the animals), but by the degree to which that potential can be realized given the constraints imposed by geography or social behaviour. Marine mammals, like birds, are of particular interest because although they are highly mobile, and hence the potential for gene flow is great, there are still clear patterns of genetic structuring (Duffield et al. 1987, Schaeff et al., In Press). What mechanisms are responsible for this and how might they play a role in structuring beluga populations?

Historical patterns of geographic isolation, local extinctions, or founder events may profoundly affect population structure. For example, belugas along the western shores of North America were completely isolated from their eastern relatives for approximately 50,000 years by the Laurentide ice sheet during the Wisconsin ice age (Denton and Huges 1981). Did these populations diverge genetically due to selection and or drift? Has there been gene flow between these populations since the ice sheet receded 7,000 years ago? Another interesting question concerns the possibility that two relict populations of belugas remained in southern habitats while the rest of the species followed the receding glaciers north to their arctic habitat. These two groups are found in the St. Lawrence estuary, Quebec and in Cook Inlet, southern Alaskan (east of the Aleutian range). This provides an opportunity to compare relict populations with their northern counterparts. For example, is the St. Lawrence population genetically similar to belugas found directly north of there in eastern Canada, and are they different from those found along western North America? Such comparisons could provide

insight into patterns of recolonization of the arctic after glacial retreat.

Local extinctions of belugas trapped in the ice and subsequent recolonization by other belugas is a process that can act to homogenize the gene pool. In 1984, a pod of 2,300 - 3,000 belugas was found trapped in the ice in the Soviet arctic (Ivashin and Shevlyagin, 1987). Although they were freed by an ice breaker, events such as this may occur periodically, wiping out local groups of belugas (Mitchell and Reeves, 1981). When new groups colonize the area, they bring with them the genetic makeup of the population they came from. Thus populations in different areas may be very similar genetically due to recent founder events.

Site fidelity, or philopatry, may play an important role in structuring beluga populations. Returning to familiar habitat with known food resources and predictable climatic conditions may be extremely important for belugas, particularly pregnant or nursing females. While belugas over-winter together in ice free bodies of water, they must migrate, often great distances, to estuaries throughout the arctic for calving, feeding, or molting in summer. Belugas enter the Churchill river the day after the ice breaks up, suggesting they were in the vicinity waiting for this to occur (Gurevich 1980). If the timing of their arrival in the estuaries is critical, there is likely to be a strong selective advantage for belugas to travel along traditional migration routes, returning to known and predictable habitat.

There is evidence that belugas do exhibit site fidelity (Caron and Smith, 1990), despite extensive seasonal migrations, mixing on the wintering grounds, and a tendency to occasionally wander quite far from "home" (Reeves and Katona, 1980). Early learning may be an important reason for this. Beluga mothers give birth after about a 14 to 15 month gestation period and suckle their calf for approximately 20 to 24 months (Brodie, 1971; Sergeant, 1973). The calving interval is thought to be roughly three years (Brodie, 1971; Sergeant, 1973). It is not uncommon to see a mother accompanied by both a neonate and an older calf from a previous season (Finley et al., 1982). Not only is there a prolonged period of maternal care, according to Gurevich (1980), "immature animals never form separate groups but remain with adult female [groups]". Studies of herd composition in the Nastapoka estuary support this (Caron and Smith, 1990). Thus there may be a period of at least five years, maybe more, where young belugas travel with their maternal group, learning the migration route, where to over-winter, and where to spend the summer. But learning probably does not stop there.

Belugas have been reported to eat over 100 different prey species (Kleinenberg et al., 1964). This includes a wide variety of fish and invertebrates, which are foraged for near the surface, mid water, or along the bottom. Belugas are excellent divers, reaching depths of over 600 meters (Ridgeway et al., 1984; Martin and Smith, 1992). This may be an important skill for food gathering in winter while in pack ice, when the only place to forage is downwards. Not only is it

important for young belugas to learn "what" to forage for "where", they must also learn "when". Seaman et al. (1982) have shown that the diet of belugas varies considerably depending on seasonal availability of particular prey species. There may also be technique or skill involved in foraging and prey capture (Hoelzel et al., 1989; Hoelzel, 1991).

Belugas must also learn about tides, currents, and bottom topography. Inuit hunters have long maintained that belugas will enter a river on a rising tide and leave on the falling tide (Finley et al., 1982). Occasionally belugas are stranded, presumably misjudging bottom topography or tides. This makes them vulnerable to predation by polar bears or hunters. Bottom composition may also be important for rubbing off skin during molting (Finley et al., 1987). Learning about local weather and ice conditions may be even more important. According to Doan and Douglas (1953) "drastic reduction in abundance of beluga [at Churchill River] was usually associated with stormy weather in late August and early September." Belugas must learn when to move out, otherwise risk ice entrapment. Subtler, yet equally important things to learn may include how to navigate in ice, determining ice conditions, thickness, cracks, etc. from the sound of returning echolocation clicks (Turl, 1990). And finally there is the need to learn complex vocal communication (Sjare and Smith, 1986) and social skills important for social interactions (Recchia and Tyack, 1991).

Much of what a beluga needs to know for survival in arctic conditions is place related. Returning to areas with reliable open water in winter is crucial (Stirling, 1980). There is probably a great selective advantage to return to a known area with known food resources, appropriate estuaries with known tides and topography, and a predictable level of risk from hunting, predation, or ice entrapment. Furthermore, social ties, important in belugas (R. Michaud, personal communication, 1991), as well as in other odontocete societies (Bigg et al., 1987; Wells, 1991; Whitehead and Weilgart, 1991), might draw belugas back to the same area. Acoustic traditions or dialects may reinforce this, as appears to be the case in killer whales (Ford and Fisher, 1982).

In conclusion, learning the skills for survival appears to occur within the maternal group. It is extremely location oriented ("forage for this here at this season", "migrate from here to there at this time"). It may have an extremely high survival value and failure to follow tradition could lead to ice entrapment, stranding, or predation. Thus there may be a strong selection pressure to follow maternally directed traditions, which are location oriented. If this is the case, a maternally inherited molecule such as mitochondrial DNA (mtDNA) could provide a neutral marker for female philopatry. MtDNA variants would thus be independent of forces selecting for philopatry but highly correlated with such behaviour. The geographic patterns of philopatry may be transmitted culturally from mother to offspring, while mtDNA is transmitted genetically from mother to

offspring. Thus mtDNA could be expected to reveal population genetic structuring of matrilines in belugas.

MtDNA is also an independent yet correlated marker for culturally transmitted maternal skills. In large brained social mammals, like primates, much of motherhood skills are learned (Harlow and Harlow, 1969; Kemps et al., 1989). This seems to be the case in odontocetes as well. Successful bottlenose dolphin mothers tend to produce daughters who are also successful mothers (Wells, 1991). Beluga mothers who successfully care for and train their young will tend to leave more offspring than those who do not. Thus in highly social mammals where there is an extended period of juvenile care and where learning is important for survival and reproduction, there may be forces other than stochastic lineage extinction that cause some mtDNA lineages to become dominant and others to disappear. This could give the appearance of a bottleneck effect or founder event when one did not necessarily occur (as has been argued for humans (Brown, 1980; Avise et al., 1984)). Together with philopatric behaviour, this might lead to greater differentiation between populations of belugas with respect to the frequency of particular mtDNA lineages.

Thus, founder events, matrilineally directed traditions, and lineage sorting due to stochastic extinction, ice entrapment or maternal influences, could be factors that act to structure beluga populations for mtDNA markers.

Beluga population structure is not just of interest to evolutionary biologists but also has important management implications. Currently there are a number of issues facing managers ranging from how to ensure the survival of the dwindling St. Lawrence population, to regulating Inuit hunting of arctic stocks depleted by previous commercial hunting, to assessing the impact of hydoelectric dams on beluga habitat usage. To make wise management decisions, it is imperative that we not only quantify beluga population structure, but more importantly, that we understand the *mechanisms* responsible for any genetic structuring observed, such as behaviour and habitat constraints.

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POPULATION GENETIC STRUCTURE OF BELUGA WHALES *(Delphinapterus leucas)*: Mitochondrial DNA Sequence Variation Within and Among North American Populations.

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INTRODUCTION

Beluga whales (*Delphinapterus leucas*) are an arctic adapted odontocete with a nearly circumpolar distribution (Gurevich, 1980). In winter, when solid ice covers much of the arctic, these air breathing mammals must seek areas with reliable polynyas or unconsolidated pack ice (Stirling, 1980; Finley et al., 1982). Occasionally ice entrapment does occur and can lead to the extermination of an entire group of whales (Ivashin and Shevlyagin, 1987; Mitchell and Reeves, 1981). When the ice breaks up in the spring, belugas disperse, sometimes traveling hundreds of kilometers to their summering grounds. During summer months belugas tend to congregate around warm shallow river estuaries. The return of belugas to particular estuaries has been reliable enough to influence the settlement patterns of arctic peoples who depend on these whales for food (Taylor, 1975). Behavioural observations have indicated that despite heavy hunting pressure, belugas will return within hours or days to the same river estuary (Caron and Smith, 1990; Finley et al., 1982). Thus it is believed these estuaries must serve some important biological function such as providing food resources (Tomilin, 1967; Kleinenberg et al., 1964) or warmer temperatures for the survival of poorly insulated neonates (Sergeant, 1973). Alternatively, the warm, low salinity estuarine water may be important for the metabolic and physiological process of molting that belugas undergo every year (St. Aubin and Geraci, 1989; St. Aubin et al., 1990). This tenacious preference for estuarine habitat has made

the beluga vulnerable to over exploitation and disturbance at a number of locations.

While traditional Inuit hunting was probably well within the level of sustainable yield, commercial harvests were not. In some areas such as Cumberland Sound (Fig. 1), as many as 800 belugas were killed in a single season (Mitchell and Reeves, 1981). Excessive commercial harvests of belugas along the southeastern Hudson Bay (Reeves and Mitchell, 1987b), Ungava Bay (Reeves and Mitchell, 1987a), Cumberland Sound (Mitchell and Reeves, 1981; Brodie et al., 1981), and the St. Lawrence Estuary (Reeves and Mitchell, 1984) during the 19th and early 20th century, severely depleted these populations. This was so severe in some locations that moderate levels of subsistence hunting appears to be driving certain beluga summer concentrations to extirpation (Richard, 1991; Reeves and Mitchell, 1989). Recovery of beluga numbers in depleted areas may be further mitigated by effects of pollution (Muir et al., 1990; Wagemann et al., 1990) or habitat destruction due to hydroelectric developments, past and proposed (Prinsenberg, 1980; Messier et al., 1986; Woodley et al., 1992).

Decisions to limit hunting or protect habitat must be based on knowledge of what constitutes a "management stock". The scientific committee of the International Whaling Commission has defined management stocks as "groups which are sufficiently isolated from neighboring groups for major changes to occur in them without affecting the adjacent stocks" (Allen, 1980). What constitutes a

beluga management stock? Is it the "group" of whales that overwinter together, or should each summer concentration be considered a separate and distinct management stock (Braham, 1984)? If belugas are philopatric, returning year after year to the same summering grounds despite mixing and possible interbreeding during winter, then each of the summer concentrations should be considered a management stock. However, mating is thought to occur in April and May (Brodie, 1971; Sergeant, 1973) while belugas are still at their common wintering ground (Finley et al., 1982). The overwintering group may represent the genetic stock, while philopatric behaviour to summering grounds may define local management stocks. Behavioural observations of marked animals suggests that belugas are site tenacious (returning after disturbance from hunters), and also philopatric (returning to the same estuary from one year to the next) (Caron and Smith, 1990).

A variety of techniques have been employed in an effort to delineate management stocks. These have included mark recapture (Sergeant and Brodie, 1969a; Sergeant, 1973), morphometrics (Sergeant and Brodie, 1969b; Finley et al., 1982), the study of geographic distribution (Sergeant and Brodie, 1975; Finley et al., 1982), and radiotagging (Frost et al., 1985). It has also been reasoned that localized population declines due to site specific over harvesting provides evidence of stock discreteness (Mitchell and Reeves, 1981). Despite these research efforts, the question has remained unanswered.

To examine this question of stock identity, we analyzed sequence variation in mitochondrial DNA (mtDNA) from belugas sampled at a number of summering locations across the arctic and subarctic. This technique is well suited to address questions of female mediated philopatry (Avise, 1987). Vertebrate mitochondrial DNA has been shown to have a high rate of mutation, on average five to ten times greater than single copy nuclear DNA, providing a high level of intraspecific variability (Brown et al., 1979). It is also inherited as a clone through the maternal line (Lansman et al., 1983), though there may be extremely small paternal transmission that goes undetected with standard techniques (Gyllensten et al., 1991). Thus variations that occur can be traced through matrilines without being obscured by recombination events. This is particularly useful for organisms where there is a high degree of matrilineal philopatry.

While analysis of mtDNA is well suited to address questions of philopatry, used exclusively it will not answer the question of what constitutes a breeding stock of belugas. Analysis of patterns of nuclear DNA variability would shed light this issue. Joint consideration of mitochondrial and nuclear DNA data would allow comparison of female versus male mediated gene flow (Lansman et al., 1981).

In this paper we expand on our earlier study (Helbig et al., 1989) to address three questions. First, is there a difference in the frequency of beluga mtDNA haplotypes among different summering areas, reflecting patterns of long-term philopatry? Secondly, is there

a marked difference in mtDNA haplotypes between the St. Lawrence and Beaufort Sea belugas, possibly reflecting divergence between an Atlantic and Pacific stock believed to have been separated for over 50,000 years during the Wisconsin Ice Age? And finally, how does the level of mtDNA sequence variation and population structure compare with that of other cetacean species and with large terrestrial mammals?

MATERIALS AND METHODS

Sample Collection

Beluga tissue samples were collected in the Hudson Bay, the Canadian arctic, and Alaska, from Inuit and Eskimo hunter kills through the generous cooperation of the Department of Fisheries and Oceans, the Fisheries Joint Management Committee, and the Department of Wildlife Management, Barrow, Alaska. The animal from Newfoundland was entangled in a fishing net. Samples from the St. Lawrence Estuary were obtained from dead animals that had washed ashore and were collected for autopsies by the Institut national d'écotoxicologie du Saint-Laurent. Tissues such as liver, muscle, kidney, and skin were collected and preserved either by freezing at -20° C or by pickling in a solution of 20% DMSO and 0.25 M EDTA saturated with NaCl (Seutin et al., 1991).

DNA Extraction

Total cellular DNA (nuclear and mitochondrial) was extracted from tissue samples either manually or using an ABI nucleic acid extractor. Hand extractions involved grinding together to a fine powder frozen tissue and lysis buffer. Liquid N_2 was poured into the mortar and pestle and refilled until it stopped boiling and the ceramic was sufficiantly cooled. Between 0.2 - 0.6 g of tissue was added to the liquid N_2 as well as 3 ml of 4 M urea, 0.2 M NaCl, 100 mM Tris-HCl (pH 8.0), 0.5% n-lauroylsarcosine, and 10 mM EDTA. Once ground, the samples were incubated at 37° C for 1-2 days. Proteinase K (65 units) was then added and samples were returned

to 37° C. After 2-5 days another aliquot of proteinase K was added to samples that were not completely digested. Each sample was extracted two or three times with one volume containing equal amounts of phenol and chloroform:isoamyl alcohol (24:1) and once with one volume of chloroform:isoamyl alcohol (24:1). DNA was precipitated by adding 0.1 volume of 3 M sodium acetate and two volumes of 95% isopropanol, chilling overnight at -20° C and centrifuging for 20 min. at 8,000 x g. The pellet was air dried and resuspended in 1 ml of TNE_2 (10 mM Tris-HCl, 10 mM NaCl, and 2 mM EDTA) by incubating at 37° C for 24 hrs.

The quantity and quality of DNA was assessed on agarose gels. Gels were stained with ethidium bromide and the DNA was visualized under shortwave UV light. Relative concentrations of mtDNA varied between samples standardized for nuclear DNA concentration because some types of tissue contain more mitochondria.

Restriction Digests, Electrophoresis, and Southern Blotting

Aliquots of total DNA (1-3 ug) were digested with 3-10 units of restriction enzyme according to manufacturer's recommendations (Bethesda Research Laboratories, Ontario, Canada). Eleven restriction enzymes recognizing sequences of four to six bases (Bam HI, Eco RI, Hind III, Bgl II, Ava II, Pst I, Kpn I, Dra I, Pvu II, Bcl I, and Hae III) were chosen randomly with care so as not to duplicate recognition sites (i.e. a 4 cutter sequence contained within a 6 base recognition site). DNA fragments were electrophoretecly separated on a 20 cm

long 1.0% agarose gel made with TAE buffer (40 mM Tris, 3 mM sodium acetate, and 1 mM EDTA, pH 7.8) and run at 33 volts 14 to 16 hrs. Gels were stained with ethidium bromide and examined under shortwave UV light, soaked for 50 min. in 0.6 M NaCl and 0.4 M NaOH to denature the DNA, and soaked for 50 min in 0.5 M Tris-HCl and 1.5 M NaCl, pH 7.5 to neutralize the DNA. The DNA was then transferred to a charged nylon membrane (either Immobilon-N® or Gene Screen Plus®) using Southern blotting technique (Southern 1975). Membranes were then air dried and baked at 80° C (1 hr. for Immobilon-N® or 2 hrs. for Gene Screen Plus®). Several blots (1-4) were prehybridized together, with a nylon mesh between each blot, in a plexiglass tube containing 30 - 60 mls of 10% dextran sulfate, 1 M NaCl, 1% SDS, and 0.1 mg/ml sheared salmon sperm DNA. The inner most layer contained a plastic sheet cut to the size of a blot to prevent drying and excessive non specific binding of the probe to the top layer blot. Blots were prehybridized for 8-16 hrs at 65° C.

Probing

In order to visualize beluga mtDNA, the complete cloned mouse mtDNA (pAM I) (Martin and Clayton 1979) was used as a probe. Homology between mice and odontocetes is reasonably high (Helbig et al., 1989; Schaeff et al., 1991). Probe DNA (25 ng per blot) was labeled with 25 μCi of [α - ^{32}P] dCTP using a random primer reaction (Feinberg and Vogelstein 1983) to a specific activity of 4 x 10^8 to 2 x 10^9 dpm/ug, and incubated with the blots, along with marker DNA (l) similarly labeled, in plexiglass tubes rotating in a hybridization oven at 65° C overnight. Blots were washed three times at 65° C in a

shaking waterbath for 10 min., 30 min., and 20 min., respectively, in a solution of 2 x SSC and 0.5% SDS. They were then autoradiographed at -70° C for 4 hrs. to 5 days using Cronex® film and a Cronex Lightning Plus® intensifying screen.

Data Analysis

Estimates of sequence divergence between mtDNA haplotype pairs were obtained from restriction-site data by Nei and Tajima's (1983) maximum likelihood method using the program DREST (version 1.0; written by L. Jin, Center for Demographic and Population Genetics, Univ. of Texas Health Sciences Center). These divergence estimates were then used to construct a dendrogram using the least squares method (Fitch and Margoliash, 1967; Cavalli-Sforza and Edwards, 1967) with the KITCH program of the PHYLIP computer package (Felsenstein, 1990). Estimates of average interpopulation genetic divergence after correction for intrapopulation divergence (gene diversity and G_{st}, after Nei (1973)) were made using a computer program provided by Lynch and Crease (1990).

RESULTS

Restriction Fragment Length Polymorphisms

MtDNA sequence variation among 101 beluga whales from 10 locations across North America and western Greenland was estimated using restriction fragment length polymorphism (RFLP) analysis. Of the eleven restriction enzymes used, one recognized four base pairs (Hae III), one recognized five base pairs (Ava II), and the rest had a six base pair recognition sequence (Bam HI, Eco RI, Hind III, Bgl II, Pst I, Bcl I, Dra I, Kpn I, and Pvu II). Seventy-one animals were tested with all 11 enzymes. The remaining 30 animals were tested with only the restriction enzymes found to identify RFLP's in order to gain a broader understanding of the geographic distribution of haplotypes. However, since only a subset of the enzymes were used, these animals were not included in estimates of sequence divergence.

Six of the eleven restriction enzymes revealed RFLP's. The most commonly found patterns generated by these enzymes are illustrated in Fig. 2, with the exception of Hae III. Each pattern generated by an enzyme was designated by a letter code A, B, or C etc. The enzymes Kpn I and Pvu II cleaved the mtDNA molecule only once (Fig. 2). The different restriction morphs A and B generated by Eco RI, Hind III, Bgl II, and Ava II could be explained by the loss or gain of a single recognition site. However, the two most common patterns generated by the enzyme Bam HI differed by two sites. The 11.0 kilobase (kb) band found in morph A gained two restriction sites and was cleaved into three bands measuring 7.0, 3.2 and 0.8 kb

in morph B. An intermediate pattern was found, morph C, (with 7.0 and 4.0 kb bands), but in only one animal.

Using these eleven restriction enzymes, the total number of fragments generated for each individual ranged between 54 and 57. Thus, a maximum of 295 base pairs were surveyed, representing 1.8% of the mtDNA molecule. The length of beluga mtDNA was estimated to be 16.3 kb (Helbig et al., 1989). From RFLP data we determined the mtDNA of the narwhal (*Monodon monocerous*), sister species of the beluga in the family *Monodontidae*, to also be 16.3 kb in length.

MtDNA Haplotypes

The letter descriptions for each enzyme restriction pattern were compiled to produce a composite restriction morph, after Lansman et al. (1983a). Nine composite restriction morphs, designated haplotype 1 through 9 were found among the 71 belugas surveyed with all 11 enzymes (Table 1). Enzymes exhibiting RFLP's are listed first. The most common haplotypes were haplotype 1 (n=32), haplotype 3 (n=6), and haplotype 5 (n=27). All other haplotypes were found in only one animal each.

A striking feature of the common haplotypes is that they fall into two groups, those represented by morph A in Bam HI, Eco RI, Hind III and Bgl II, and those represented by morph B for the same enzymes. The nine haplotypes cluster into two major groups in a phylogenetic dendrogram (Fig. 3). We have labeled the first group "lineage I" (composed of haplotypes 1 and 2) and the second group

"lineage II" (composed of haplotypes 3 through 9). There is relatively little variation within lineage I compared with lineage II. Lineage I has only two haplotypes, of which haplotype 2 was only found in one animal.

Estimates of sequence divergence between pairs of haplotypes, based on the site method (Nei and Tajima, 1983), are given in Table 2. The proportion of shared fragments (F) between different haplotypes are given in the lower left matrix of Table 2. These values were used to estimate the average number of nucleotide substitutions per site (δ) separating the different haplotypes. These values are given in the upper right matrix of Table 2. The average nucleotide diversity among all 71 beluga whales was estimated to be 1.01% after Nei and Li (1979). From the phylogenetic dendrogram (Fig. 3), it can be seen that lineage I and lineage II are separated by 1.?? % sequence divergence. Thus, haplotypes 1 and 2 in lineage I are quite divergent from all other haplotypes in lineage II.

Geographic Distribution of Haplotypes

There is a distinct pattern of geographic distribution of haplotypes according to whether they belong to lineage I or lineage II (Fig. 4). Lineage I haplotypes were found primarily in the St. Lawrence Estuary and eastern Hudson Bay animals. One stray animal caught in a fishing net off Chance Cove, Newfoundland was also lineage I. While eastern Hudson Bay was composed almost entirely of lineage I belugas (19 out of 21 animals), western Hudson Bay was composed mainly of lineage II (19 out of 20 animals). Belugas

captured in Hudson Strait (n=7), the common wintering ground, were composed of both lineage I and II in roughly equal proportions. Yet all other locations ranging from southern Baffin Island, western Greenland, Grise Fjord in the high arctic, to the Mackenzie Delta and Alaska were composed entirely of animals from lineage II.

A more detailed breakdown of the frequency distributions of haplotypes 1 through 9 for most geographical locations is given in Fig. 5. Eastern Hudson Bay is composed primarily of haplotype 1, whereas western Hudson Bay is composed mostly of haplotype 5. The Mackenzie Delta, on the other hand, has five different haplotypes of approximately equal frequency, and therefore has more intrapopulation variation.

The sex of each animal was determined upon examination of the carcass at the time of tissue sample collection. There was no significant sex bias in the geographic distribution of haplotypes indicating that if there is a sex-specific difference in dispersal, it must be minor and does not confound these results.

Population Differences

We compared 4 eastern Canadian beluga summer populations, calculating the gene diversity between populations, after correction for intrapopulation diversity (Lynch and Crease, 1990). In this calculation only the relative frequencies of mtDNA haplotypes were considered. These values, along with their associated standard error, are given in Table 3. Diversity is low between the eastern Hudson Bay

and St. Lawrence Estuary populations, and between the western Hudson Bay and southern Baffin Island populations. However, the eastern and western Hudson Bay populations are quite divergent (gene diversity = 0.7281), as is graphically illustrated in Fig. 4. We also calculated interpopulation sequence divergence between eastern and western Hudson Bay populations taking into account both the sequence divergence between haplotypes and their relative frequencies in each population (Nei and Li, 1979). Sequence divergence was estimated to be 0.59% within eastern Hudson Bay and 0.44% within Western Hudson Bay. Sequence divergence between these two populations, after correcting for the intrapopulation sequence divergence, was calculated to be 3.15%.

The largest sample sizes in this study were drawn from both sides of the Hudson Bay with the intention of addressing the question of whether there is one intermingling population within the bay or if belugas are philopatric, homing on traditional summering locations. There is a distinct pattern in the geographical distribution of mtDNA haplotypes within the bay. In the eastern Hudson Bay, 19 out of 21 belugas sampled were haplotype 1 (the other two were haplotype 3 and 5). In the western Hudson Bay, 18 out of 20 animals were haplotype 5, one was haplotype 6 (a unique variant of 5), and only one animal was haplotype 1. To test the significance of the distribution of the four beluga haplotypes among 41 whales within the Hudson Bay we used the χ^2 test. We found that there is a significant non-random distribution of haplotypes between east and west coast ($p<0.001$).

DISCUSSION

MtDNA Population Structure

To clearly understand the population genetic structure of beluga whales in eastern Canadian waters, two questions must be addressed. First, do animals move between different summering grounds, or is each local estuarine concentration a distinct group that faithfully returns to that area despite intermixing and possible interbreeding on the common wintering ground? And secondly, what constitutes the breeding population? Is it the group that overwinters together, or does each summer concentration constitute a distinct breeding stock? The mtDNA data presented here allows us to address the first question. Future studies, we hope, will address the second one. For lack of better terminology, here we use the term "management stock" to refer to a distinct group of whales in which the exchange of individuals with other groups is minimal, and we use the term "genetic stock" to refer to the breeding population.

Belugas from the eastern and western Hudson Bay, Southern Baffin Island, and Ungava Bay are thought to overwinter together in Hudson Strait (Fig. 1) (Finley et al., 1982). The relationship of these summering groups to one another has been unclear. Earlier work has shown that Cumberland Sound belugas are significantly larger then western Hudson Bay animals, suggesting that they constitute a separate genetic stock (Sergeant and Brodie 1969b). However, it seems that Ungava Bay, Hudson Strait, eastern and western Hudson Bay belugas cannot be distinguished on the basis of size (Finley et al.,

1982). Furthermore, recent aerial surveys have shown that there is a near continuous distribution of beluga whales around the perimeter of the Hudson Bay (Richard et al. 1990). Reeves and Mitchell (1987a) developed four hypotheses concerning management stock affinities in eastern Canadian waters:

1. the whales throughout Hudson and James bays, Foxe Basin, Hudson Strait and Ungava Bay and from Cumberland Sound to central Labrador belong to a single [management] stock;
2. animals summering in Ungava Bay, eastern Hudson Bay, western Hudson Bay, Cumberland Sound and possibly James Bay and Frobisher Bay should be regarded as separate [management] stocks;
3. there are only two [management] stocks: one summering in Cumberland Sound and Frobisher Bay, the other in Ungava Bay, Hudson Strait and Hudson Bay;
4. there are many different [mangement] stocks, each recognized by its tendency to "home" in summer on a particular estuary or group of estuaries.

The mtDNA data may help clarify this issue. We have found a significant difference ($p<0.001$) in the frequency distribution of mtDNA haplotypes between eastern and western Hudson Bay. This is striking given that these animals are believed to intermix, and possibly interbreed, on the common wintering ground in Hudson Strait. Despite this, these animals appear to consistently return to their specific summering grounds. Even though the geographic distance between these summering grounds is equal to or less than

their annual seasonal migration, there appears to have been remarkably little exchange of individuals. This suggests that such philopatric behaviour must serve some important function for belugas.

In the fall, as ice excludes belugas from most of their range, it is crucial that they return to areas with known open water. Philopatry to wintering grounds helps belugas avoid ice entrapment. The Hudson Strait samples were collected in the fall and are believed to represent migratory rather than resident animals (St. Aubin and Geraci, 1989; D. St. Aubin, personal communication, 1992). The fact that both lineage I and lineage II haplotypes were found among belugas from Hudson Strait supports the hypothesis that this is indeed a common wintering ground. However, it is not clear if the lineage II belugas captured in Hudson Strait are from western Hudson Bay or from southern Baffin Island, or both. What is clear is that there is also remarkably little exchange between eastern Hudson Bay and southern Baffin Island. Indeed, the interpopulation genetic difference (N_{ST}) between the eastern Hudson Bay belugas and those in western Hudson Bay (0.728) and southern Baffin Island (0.469) is high.

The mtDNA evidence presented here is consistent with the hypothesis that belugas are philopatric with respect to summering grounds, and that animals from different summering grounds do overwinter together in Hudson Strait, thus providing the opportunity for interbreeding between these groups. Together with the

morphometric data, it seems that hypothesis 1 and 3, put forth by Reeves and Mitchell (1987a), should be rejected in favor of either 2 or 4, i.e. that each summering concentration should be regarded as a distinct management stock, but not necessarily a distinct breeding stock.

The tendency to home on natal summering grounds does not rule out movement between areas during migration or by pods that remain offshore and hence are not represented in our estuary based hunter kill samples. Thus animals hunted offshore during the migration might not belong to the management stock of that area. Richard et al. (1990) discussed evidence that during spring migration, western Hudson Bay belugas leave Hudson Strait heading south along the east coast. Local aerial sightings confirm this (Denis Ladouceur, personal communication, 1991). Furthermore, a portion of the population may not visit estuaries on a regular basis (Richard et al., 1990). Surveys along the eastern Hudson Bay revealed that 68 - 80% of the belugas censused were more than 10 km from shore (Smith and Hammill, 1986). However, Smith and Hammill (1986) found no significant differences in the age structure between inshore versus offshore groups, and suggest that there may be continuing exchange between these groups throughout the summer.

We wondered if there was a sharp division between the distribution of lineage I and lineage II haplotypes somewhere along the south coast of the Hudson Bay, or if estuaries in that region contain a mixture of both lineages. We therefore attempted to obtain

samples from belugas within the Winisk River (Fig. 1) using a crossbow and biopsy dart but were unsuccessful (Brennin, 1992).

Historical Biogeography

The striking split of genetically divergent lineages between the east and west coast of Hudson Bay may be maintained by the philopatric behaviour of belugas. But what explains the origin of such a distribution? The geological history of North America might provide clues. Belugas on both sides of the North American continent are thought to have been separated for over 50,000 years by the Laurentide Ice Sheet during the Wisconsin Ice Age (Denton and Hughes, 1981). Roughly 9,000 years ago permanent ice retreated from the high arctic enough to create a connection between the Beaufort Sea and Baffin Bay, but it was not until 8,000 years ago that the Hudson Bay began to open up (Dyke and Prest, 1987). During the period of separation, Atlantic and Pacific belugas may have diverged in their mtDNA as a consequence of stochastic lineage extinction (Avise et al., 1984), a process that may be accelerated in belugas due to ice entrapment. This is not uncommon (Mitchell and Reeves, 1981), and as many as several thousand individuals have been entrapped at once (Ivashin and Shevlyagin, 1987). Lineage I and lineage II may represent the end result of such sorting in the Atlantic and Pacific "refugia" respectively. The fact that the St. Lawrence Estuary sample was composed entirely of lineage I belugas while the Beaufort Sea sample was composed entirely of lineage II animals is consistent with this hypothesis.

Given that belugas appear to be philopatric, the geographic structuring of mtDNA we see today might represent the approximate pattern of recolonization of the arctic by Atlantic and Pacific stocks. High arctic, western Greenland, and southern Baffin Island belugas might represent the first stage of colonization by Beaufort Sea animals. Lineage I belugas living off the coast of Labrador could have colonized eastern Hudson Bay through Hudson Strait about 8,000 years ago (Dyke and Prest, 1987). A.S. Dyke (personal communication, 1992) has suggested that western Hudson Bay may have been colonized around the same time by lineage II belugas from the Beaufort Sea, entering the bay through Rae Isthmus, then submerged between Keewatin and Melville Peninsula (Fig. 1). Alternatively, they may have entered through Fury and Hecla Strait about 7,000 years ago (A.S. Dyke, personal communication, 1992). While belugas have a poor fossil record, evidence from radiocarbon-dating of bowhead whale fossils indicates that such patterns of colonization of arctic waters by these cetaceans immediately followed deglaciation (Dyke and Morris, 1990).

Although much of this is speculative, it has implications to the issue of stock discreteness. If belugas in eastern and western Hudson Bay originated from two genetically different populations that were also culturally divergent, it might help explain why there has been relatively little interchange between them over the past 7,000 years. Three sympatric communities of killer whales (*Orcinus orca*) living in British Columbia and Washington State waters have been shown to have distinct acoustic traditions (Ford and Fisher, 1982). These three

groups have never been observed to mix (Bigg et al., 1987) and may have distinct mtDNA (Stevens et al., 1989). The possibility exists that cultural traditions other than philopatry play a role in structuring beluga populations. Although belugas exhibit complex vocalizations (Sjare and Smith, 1986), at this point it is not possible for researchers to discern dialect differences (B.L. Sjare, personal communication, 1987).

Genetic Diversity

An important aspect of beluga genetic structure is that there are two very divergent lineages. Much of the diversity among beluga whales (1.01%) arises from the sequence diversity between these two lineages. For example, divergence between haplotype 1 (lineage I) and haplotype 7 (lineage II) is as high as 5.2%. Among lineage I belugas (n=33) only two haplotypes were found, while among lineage II belugas (n=38) seven haplotypes were found. Thus the diversity within lineage I is low. Geographic locations where lineage I belugas predominate (St. Lawrence Estuary and eastern Hudson Bay) are also areas where beluga populations have been depleted due to over harvesting (Reeves and Mitchell, 1984; Reeves and Mitchell, 1989). Lineage II haplotypes predominat in regions with large beluga populations, presently as well as historically (Richard et al., 1990; Finley et al., 1987), with the exception of Cumberland Sound (Richard, 1991). This is consistent with the suggestion that population bottlenecks may substantially decrease mtDNA variability (Wilson et al., 1985).

The level of sequence divergence for belugas (1.01%) appears to be high in comparison with humpback whales and right whales. Baker et al. (1990) estimated a sequence divergence of 0.25% among 84 humpback whales (*Megaptera novaeangliae*) from two oceans, while among 10 southern right whales (*Eubalaena australis*) sequence divergence was estimated to be 0.24% (Schaeff et al., 1991). However, both these species have recently experienced a population bottleneck as a consequence of whaling operations. A measure of sequence divergence between species should be viewed with caution when either or both species have gone through a population bottleneck. During such an event, stochastic sorting of mtDNA lineages could lead to an inflated or misinterpreted level of interspecific sequence divergence. For example, divergence between the north and south Atlantic right whale in the genus *Eubalaena* was found to be 1.82% and was given as evidence, together with geographic discontinuity, that these two groups represent separate species (Schaeff et al., 1991). Given that the divergence between the east and west Hudson Bay belugas was found to be 3.39%, it appears that this level of divergence can be found within the same cetacean species. Philopatric behaviour may lead to populations highly structured for divergent mtDNA haplotypes, but does not preclude the possibility that gene flow of nuclear DNA may still occur.

Belugas, like humpback whales (Baker et al., 1990) and right whales (Schaeff et al., In Press), appear to exhibit population structuring for mtDNA haplotypes. In all three species this seems to be a consequence of maternally directed philopatry to summering

grounds. However, large terrestrial mammals such as bears (Cronin et al., 1991) and jackals (Wayne et al., 1990), shown to have a high level of sequence diversity, exhibit relatively little population structuring for mtDNA. These studies conclude that "phylogenetic relationships of haplotypes and their geographic distribution are discordant" (Cronin et al., 1991), and that this "may reflect unique dispersal abilities of large carnivores" (Wayne et al., 1990). However, cetaceans have an even greater capacity for dispersal and live in an environment with relatively few geographic barriers. While terrestrial carnivores may increase their chances of finding prey through dispersal, food resources in the aquatic environment often exhibit a clumped distribution around areas of high productivity, such as undersea mounts or banks. Physical habitat features such as polynyas or shallow estuaries may also define within specific limits where belugas do and do not go. Thus returning to specific areas with known resources may be more important for mammals in an aquatic environment. In such species, measures of mtDNA interpopulation diversity should be viewed with caution, as they are an indication of the movement of animals and not necessarily the movement of nuclear genes.

Management Implications

The mtDNA evidence presented here supports the hypothesis that belugas are philopatric with respect to their natal summering grounds despite extensive seasonal migrations and mixing on the wintering grounds. This has important implications for management issues. Such issues include the subsistence hunt on depleted stocks in

Cumberland Sound (Richard, 1991), Ungava Bay and eastern Hudson Bay (Reeves and Mitchell, 1989), and hydroelectric developments proposed for the Little Whale and possibly Nastapoka rivers (Fig. 1) (Woodley et al., 1992). Local beluga concentrations should be considered distinct management stocks. The philopatric behaviour of belugas implies that when a local management stock is depleted, belugas from another area will not change their migration patterns easily in order to recolonize the depleted area. Loss of a local population represents the loss of culturally transmitted traditions. The issue of whether each management stock also represents a distinct genetic stock is unclear. Analysis of patterns of nuclear DNA variability among belugas may clarify this. The issue is an important one, for if management stocks represent genetic stocks, extirpation would result in a loss of both cultural and genetic information.

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Table 1. Composite restriction morphs found among 71 beluga whales from 8 locations.

	Bam HI	Eco RI	Hind III	Bgl II	Ava II	Hae III	Pst I	Bcl I	Dra I	Kpn I	Pvu II
1.	A	A	A	A	A	A	A	A	A	A	A
2.	A	A	A	A	C	_ [1]	A	A	A	A	A
3.	B	B	B	B	A	D	A	A	A	A	A
4.	B	B	B	A	A	D	A	A	A	A	A
5.	B	B	B	B	B	B	A	A	A	A	A
6.	B	B	B	B	B	C	A	A	A	A	A
7.	B	B	B	B	B	E	A	A	A	A	A
8.	B	B	B	B	B	D	A	A	A	A	A
9.	C	B	B	B	B	D	A	A	A	A	A

[1] The one sample exhibiting haplotype 2 had DNA that was too sheared to be able score all the fragments resulting from Hae III digestion.

Table 2. Estimated sequence divergence (base substitutions per nucleotide) between nine beluga mtDNA haplotypes. Above the diagonal: sequence divergence among pairs of haplotypes, weighted by classes of restriction enzymes (Nei and Tajima, 1981; Nei and Tajima, 1983; Nei, 1987). Below the diagonal: fraction of shared restriction sites.

	1	2	3	4	5	6	7	8	9
1	-	0.005	0.027	0.021	0.041	0.049	0.051	0.033	0.031
2	0.97	-	0.033	0.027	0.043	0.050	0.052	0.034	0.033
3	0.87	0.85	-	0.005	0.012	0.018	0.020	0.005	0.011
4	0.90	0.87	0.97	-	0.018	0.025	0.026	0.011	0.017
5	0.81	0.80	0.94	0.91	-	0.005	0.003	0.007	0.013
6	0.78	0.77	0.91	0.88	0.97	-	0.013	0.013	0.018
7	0.77	0.77	0.90	0.87	0.98	0.94	-	0.014	0.020
8	0.85	0.84	0.97	0.95	0.96	0.94	0.93	-	0.005
9	0.85	0.85	0.95	0.92	0.94	0.91	0.90	0.97	-

Table 3. Gene diversity between beluga populations: St. Lawrence Estuary (St. Law), eastern Hudson Bay (East Hud.), western Hudson Bay (West Hud.), and southern Baffin Island (South Baffin). Estimates of gene diversity between populations after correction for intrapopulation diversity are given in the upper right matrix. Associated estimates of standard error are given in the lower left matrix. The proportion of genetic variability attributable to population differentiation (G_{st}) = 0.549 (SE=0.026). The D-statistic for test of between population heterogeneity = 349.54 ($p<0.01$) (after Lynch and Crease, 1990).[1, 2, 3]

	St. Law.	East Hud.	West Hud.	South Baffin
St. Lawrence Estuary	-	0.008	0.747	0.489
Eastern Hudson Bay	0.023	-	0.722	0.469
Western Hudson Bay	0.121	0.125	-	0.143
Southern Baffin Island	0.117	0.114	0.202	-

[1] An N_{st} value of 0 indicates no population subdivision, while a value of 1 indicates complete population subdivision.

[2] Calculations based on haplotype frequencies only and did not take into account sequence divergence between haplotypes.

[3] Estimates of standard error take into account 2 possible sources of sampling error: sampling of haplotype frequencies and sampling of nucleotide sites.

Figure 1. Map of the eastern Canadian arctic, showing place names mentioned in the text and locations from where eastern Canadian samples were obtained. Sample size at each of the following locations were: Nastapoka River (n=21), Churchill River (n=16), Arviat (n=4), Wakeham Bay, Hudson Strait (n=7), Cumberland Sound (n=9), and Frobisher Bay (n=4).

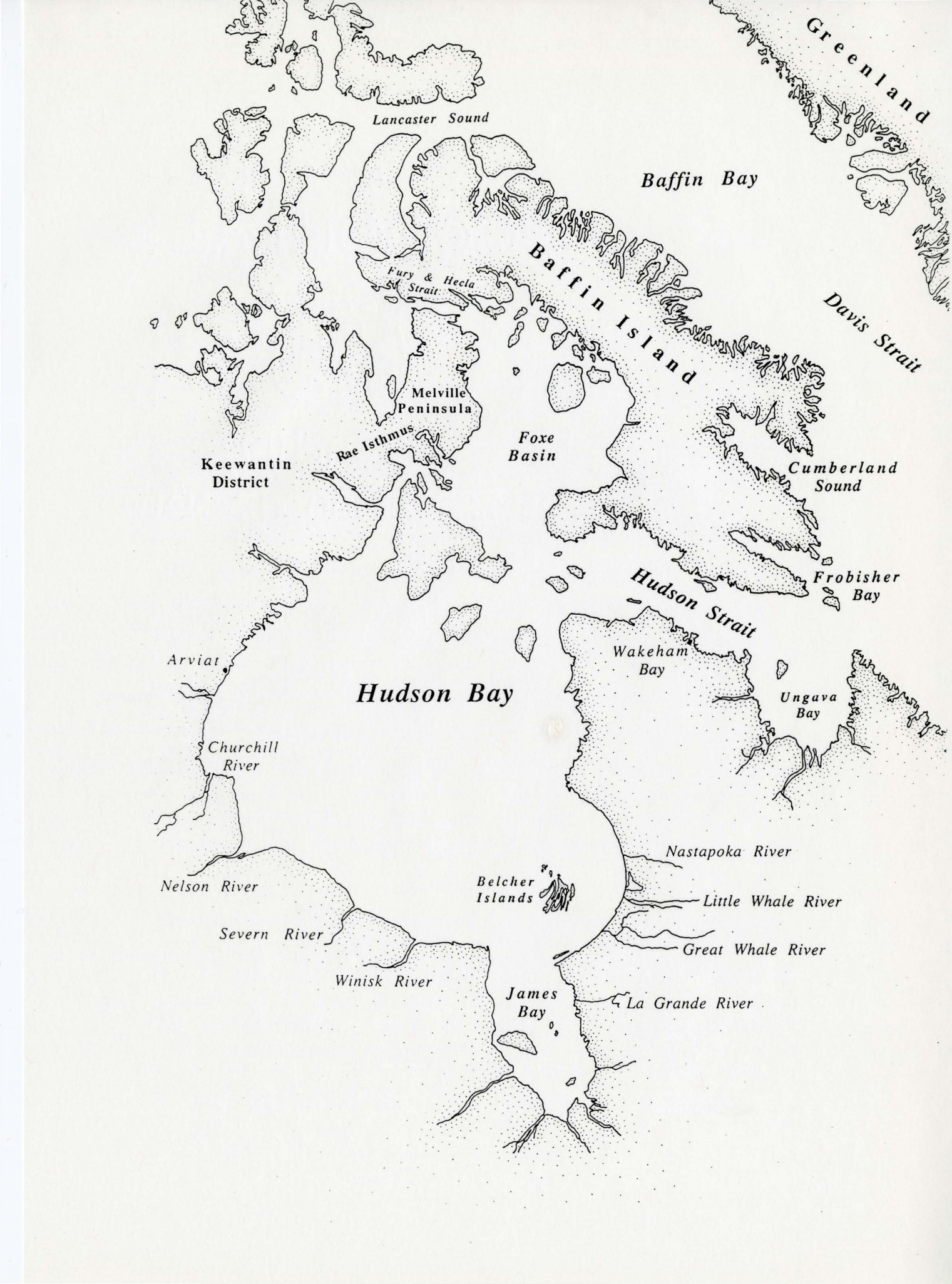

Greenland
Lancaster Sound
Baffin Bay
Fury & Hecla Strait
Baffin Island
Davis Strait
Melville Peninsula
Rae Isthmus
Foxe Basin
Keewantin District
Cumberland Sound
Frobisher Bay
Hudson Strait
Wakeham Bay
Arviat
Hudson Bay
Ungava Bay
Churchill River
Nastapoka River
Nelson River
Belcher Islands
Little Whale River
Severn River
Great Whale River
Winisk River
James Bay
La Grande River

Figure 2. Autoradiograph showing beluga mtDNA fragments generated by ten restriction enzymes. Five enzymes produced more than one restriction morph. The most common morphs, labeled A and B, are shown. The other five enzymes generated only one pattern, labeled A. Restriction enzymes, from left to right are Bam HI (Ba), Eco RI (Ec), Hind III (Hi), Bgl II (Bg), Ava II (Av), Pst I (Ps), Bcl I (Bc), Dra I (Dr), Kpn I (Kp), and Pvu II (Pv). Size markers (lanes "M") consisted of co-electrophoresed Hind III/Eco RI and Hind III digests of λ DNA.


Ba Ec Hi Bg Av Ps Bc Dr Kp Pv
M A B A B A B A B A B M A A A A A

Figure 3. Phylogenetic dendrogram of beluga mtDNA haplotypes. The dendrogram was constructed with the Kitsch computer program using divergence estimates (Table 2) of beluga mtDNA.

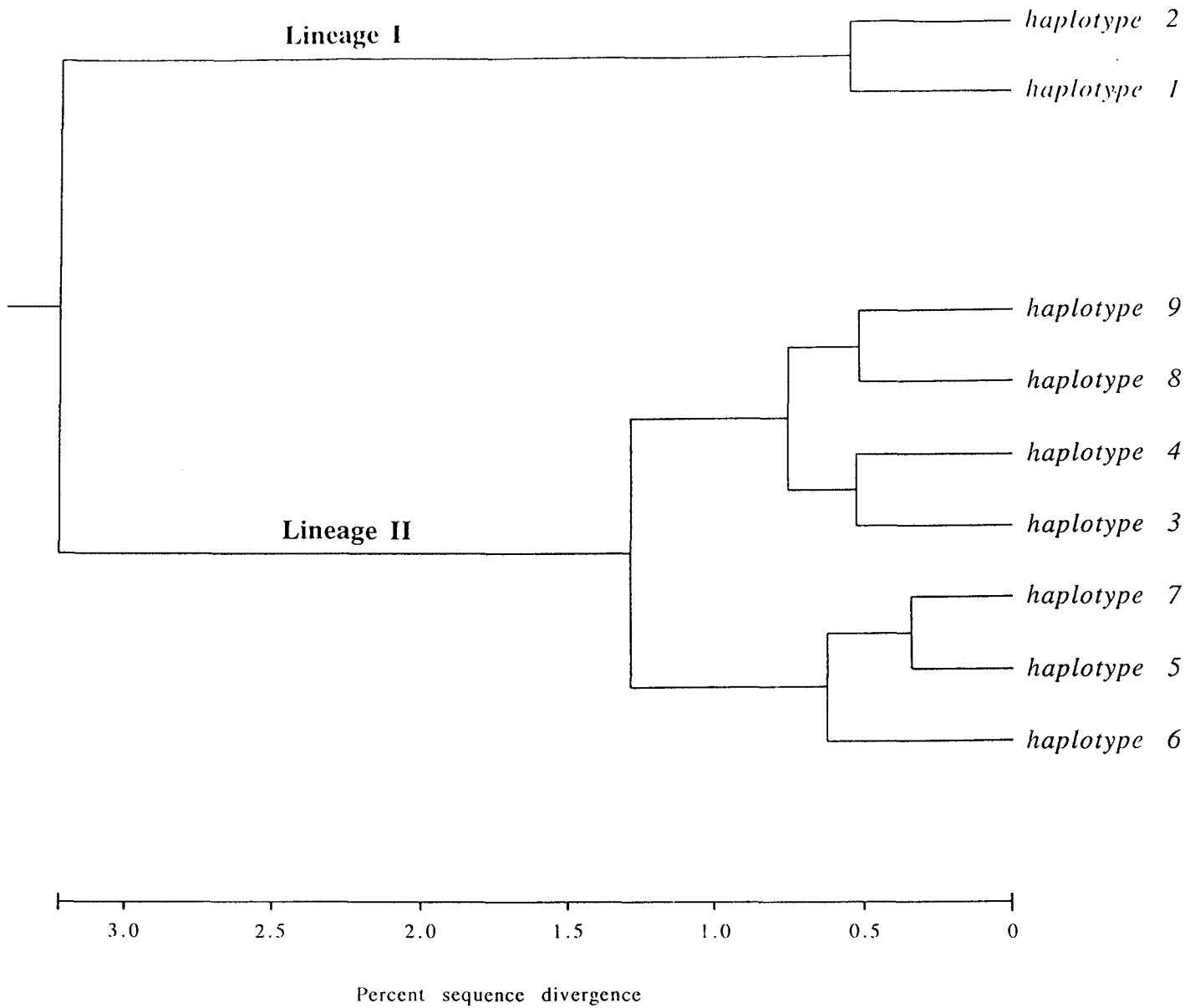

Lineage I
haplotype 2
haplotype 1
haplotype 9
haplotype 8
haplotype 4
haplotype 3
Lineage II
haplotype 7
haplotype 5
haplotype 6
3.0
2.5
2.0
1.5
1.0
0.5
0
Percent sequence divergence

Figure 4. Geographic distribution of beluga mtDNA lineage I haplotypes (shaded) and lineage II haplotypes (black). Total area of pie chart represents approximate sample size at each of the following locations: 1. St. Lawrence Estuary (n=9), 2. Chance Cove, Newfoundland (n=1), 3. Wakeham Bay, Hudson Strait (n=7), 4. Nastapoka River, eastern Hudson Bay (n=21), 5. Churchill River and Arviat, western Hudson Bay (n=20), 6. Cumberland Sound and Frobisher Bay, southern Baffin Island (n=13), 7. western Greenland (n=4), 8. Grise Fijord (n=4), 9. Mackenzie Delta (n=12), and 10. Alaska (n=4).

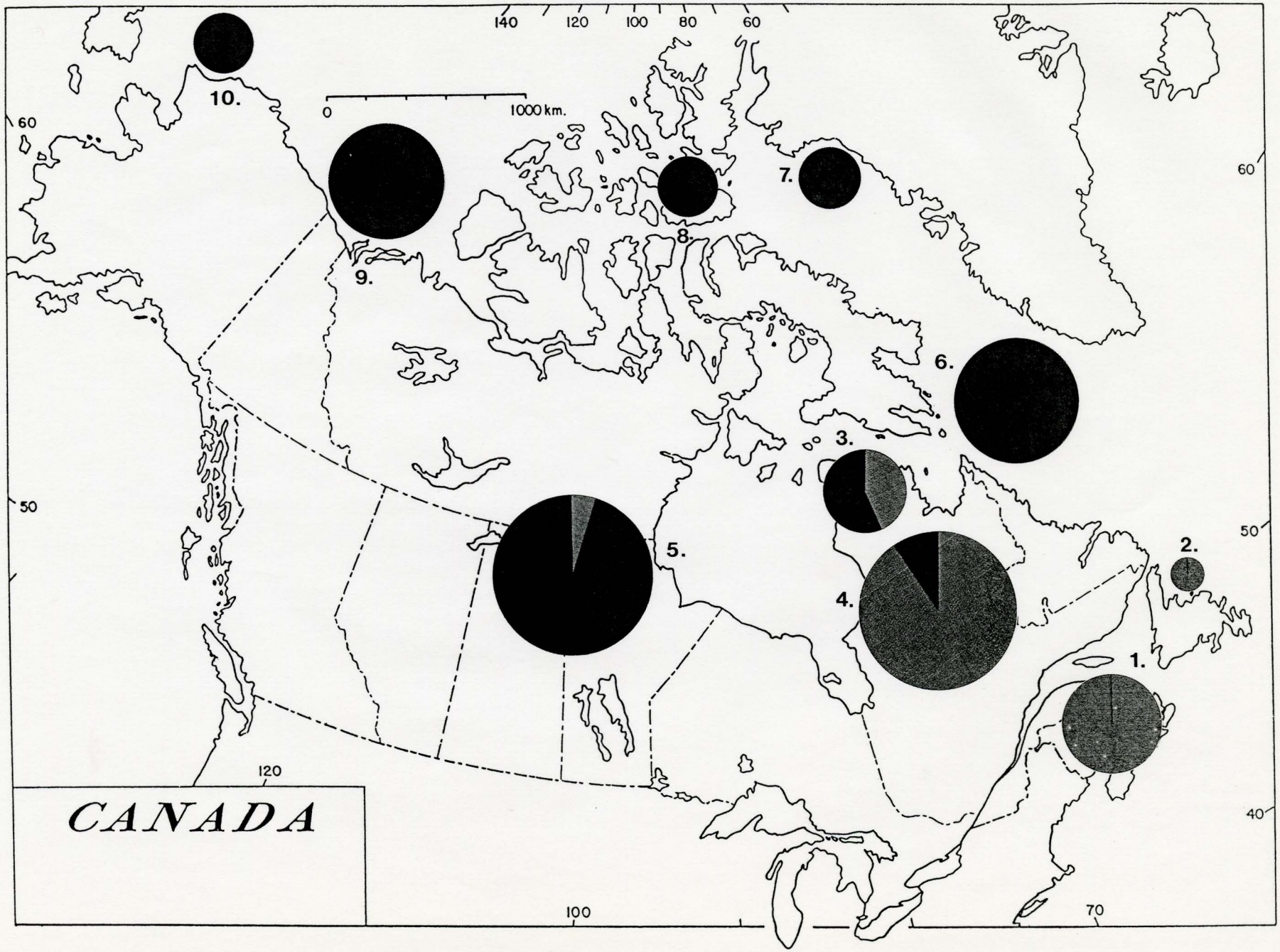
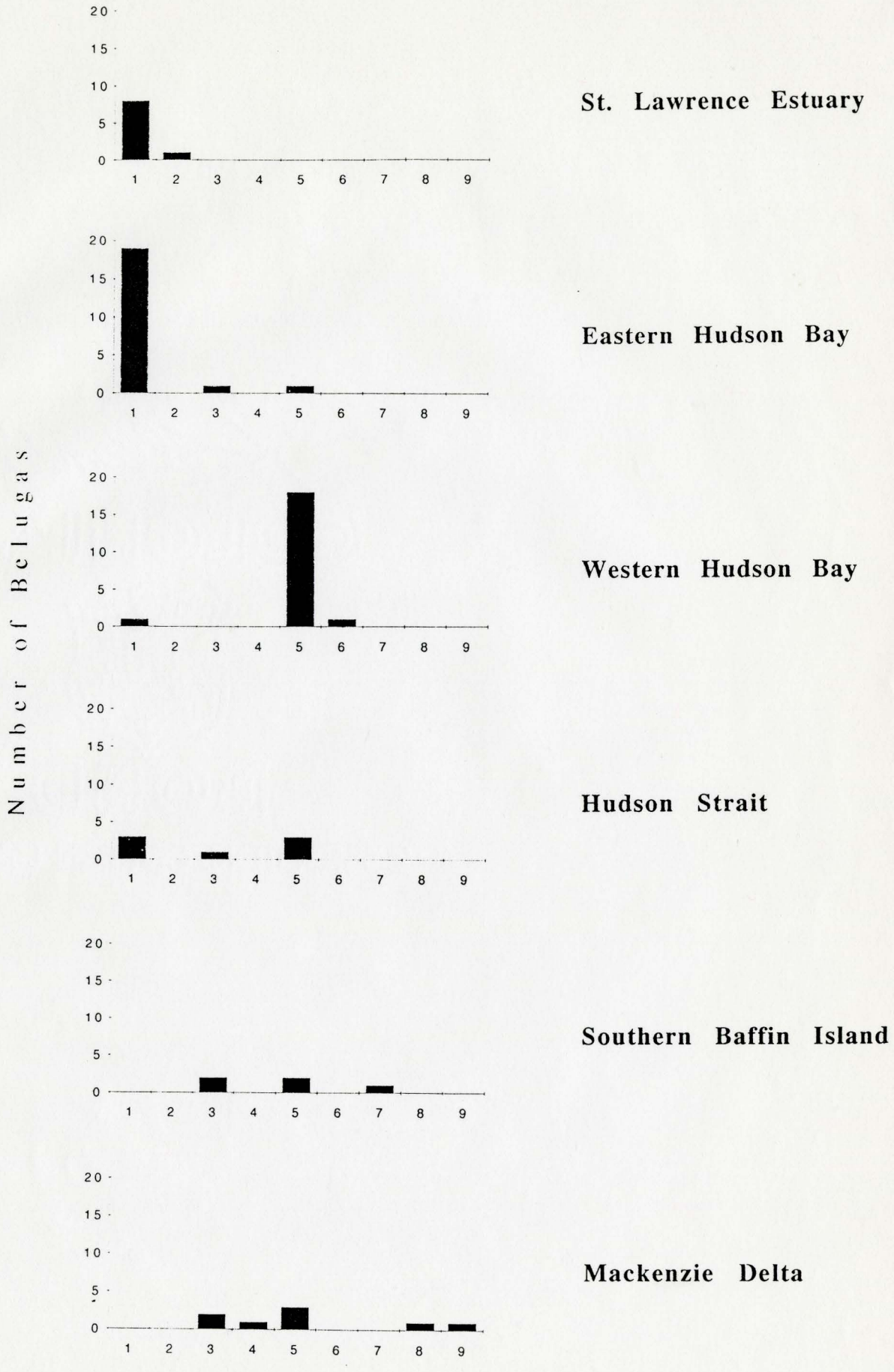

140
120
100
80
60
0
1000 km.
60
50
120
100
70
40
10.
9.
8.
7.
6.
5.
4.
3.
2.
1.
CANADA

Figure 5. Frequency distributions of beluga mtDNA haplotypes 1 to 9 among geographical regions.


St. Lawrence Estuary
Eastern Hudson Bay
Western Hudson Bay
Hudson Strait
Southern Baffin Island
Mackenzie Delta
Number of Belugas
Haplotype
0
5
10
15
20
1
2
3
4
5
6
7
8
9

CONCLUSION

The mtDNA data, taken together with other evidence such as morphometric data discussed above, suggests that eastern Hudson Bay, western Hudson Bay, and Cumberland Sound belugas should all be considered separate management stocks, even though they may share a common wintering ground. We were unable to obtain samples from the severely depleted, possibly extirpated, group in Ungava Bay. This is unfortunate, for we do not know if they too could be distinguished genetically as a separate management stock. The irony is that its depletion is evidence that it was distinct, and should have been managed more prudently.

Although we can reject Reeves and Mitchell's (1987a) hypothesis 1 and 3 in favour of hypothesis 2: "animals summering in Ungava Bay, eastern Hudson Bay, western Hudson Bay, Cumberland Sound, and possibly James Bay and Frobisher Bay should be regarded as separate [management] stocks", we cannot yet discriminate between this and hypothesis 4: "there are many different [management] stocks, each recognized by its tendency to 'home' in summer on a particular estuary or group of estuaries". Yet two examples, the Great Whale River and Ungava Bay (Fig. 1), are worth considering. Reeves and Mitchell (1987b) estimated that there were some 6,600 belugas inhabiting the Great Whale and Little Whale rivers in the 1800's. After commercial harvesting in the 1850's and 1860's, the population steadily declined. No major concentration can now be found in the Great Whale River, despite extensive surveys

(Smith and Hammill, 1986; Breton-Provencher, 1980). Why have whales from the Nastapoka and Little Whale rivers not immigrated there? Likewise, belugas in Ungava Bay have been so extensively hunted that no population estimate can be made based on the few individuals sighted there (Smith and Hammill, 1986). Since thousands of belugas overwinter in the Hudson Strait, Ungava Bay region, why have no other whales immigrated to Ungava Bay for the summer? The answer, it seems, is that matrilineally directed traditions may be so strong that once an estuarine stock has been exterminated, it will take a very long time before belugas from another estuarine stock are willing to change their traditions in order to recolonize the depleted area. This has profound implications for management.

The striking geographic distribution of mtDNA haplotypes within the Hudson Bay suggests that it may have been colonized by belugas originating from two mitochondrially divergent, and possibly culturally divergent, stocks. MtDNA is an independent yet highly correlated marker for maternally transmitted cultural traditions. Adherence to tradition, particularly philopatry, may have discouraged the exchange of animals between these groups and their respective summering grounds. Learning and following cultural traditions may be extremely important for the survival of air breathing marine mammals like belugas in harsh arctic conditions. Patterns of ice cover and subsequent breakup dictate, within narrow limits, exactly where and when belugas can utilize certain portions of their habitat for feeding, calving, or molting. With narrow seasonal

windows within which to carry out these important biological functions, it is crucial that belugas faithfully adhere to traditions of where to go when to do what. These traditions likely represent the accumulated experiences of surviving belugas over countless generations. Therefore, estuaries that are over hunted will not simply be replenished by belugas from other areas. Belugas displaced by changes in critical habitat due to hydroelectric developments will not simply move on down the line to other estuaries.

Hydroelectric dams have already been constructed along La Grande River and are slated for the Great Whale, Little Whale, and possibly Nastapoka rivers (Fig. 1) (Woodley et al., 1992). The operation of such dams significantly alters the temperature, volume and timing of river outflow (Prinsenberg, 1980). Thus the temperature, salinity, depth, and currents within these estuaries would be altered. At the very least, this would invalidate the accumulated knowledge belugas have of the local region. At worst, such changes could eventually render those estuaries and surrounding areas unsuitable for calving, molting and feeding. Of immediate concern, however, is that such changes may alter the pattern and timing of ice cover. Spring breakup may occur later, fall freeze up may occur earlier (Woodley et al., 1992). The location and availability of polynyas, or leads in the ice, important for beluga migration and survival, may be significantly altered. Therefore, the narrow window of time that critical habitat is available to belugas would become even shorter. Following traditions of where and when

to migrate, invalidated by changes in hydrology, could lead to ice entrapment and death of belugas.

Wildlife management has entered a new era. Insights gained from molecular genetics, telemetry, and behavioural studies make it clear that wildlife management is not just a numbers game. It is not enough to say there are this many animals in this kind of habitat. If the habitat is suddenly altered, it may invalidate traditions critical for survival. If older animals are suddenly killed off, important cultural knowledge may be lost. Therefore, social behaviour and cultural traditions in higher animals must be considered if management is to be effective.

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APPENDIX I

PILOT PROJECT TO ASSESS BIOPSY SAMPLING OF BELUGA WHALES IN THE WINISK RIVER ESTUARY, July 14 - 24, 1991

Ree Brennin

INTRODUCTION

There is concern that the St. Lawrence beluga whales represent a genetically isolated and inbred population. Inbreeding depression may be a factor responsible for the failure of the population to increase despite the fact that it has been protected since 1978. To test this, we have been analyzing DNA samples collected from dead stranded St. Lawrence belugas using the major histocompatibility (MHC) locus and DNA fingerprints as genetic markers. There are two difficulties inherent in relying on samples collected from dead stranded animals. The first is the sporadic and infrequent occurrence of fresh carcasses washing ashore, resulting in a very small sample size. The second is that the MHC locus is involved in the immune response. Animals with low variability in their MHC locus are more likely to show a poor immune response and thus be more susceptible to disease and death. Relying only on samples collected from dead animals may bias the results. To remedy this, we would like to collect samples from live belugas in the St. Lawrence Estuary.

To test the feasibility of this we first tried the procedure on belugas in the Winisk River Estuary along the southern coast of the Hudson Bay. This work was part of a joint research project conducted by the Department of Fisheries and Oceans (DFO), McMaster University, and the Ontario Ministry of Natural Resources (OMNR) during July 14 - 24, 1991. The first week was devoted to aerial surveys and the last few days of the study were available for biopsy work. However, due to bad weather we were only able to get out on the water one day. From the experience gained here we have developed several recommendations concerning the logistics of collecting skin samples from St. Lawrence beluga whales.

EXPERIENCE IN WINISK

There are two aspects of the biopsy procedure that must be considered, the equipment used to collect the skin sample, and the reaction of the belugas, both to the boat and the biopsy. We used three types of equipment, an Astro Daco model 1000 crossbow with a 150 lb. draw weight, a Jennings Devastator model compound crossbow with an adjustable draw weight set at 100 lbs., and a jab stick made from an aluminium telescoping pole with a biopsy tip attached to the end of it.

In order to get a shot or jab at the exposed back of a beluga, we had to make a fairly close approach with our boat (a 14.5 foot Mark II zodiac with a 9.9 horsepower Johnson outboard motor). Although beluga whales in the Winisk River have not been hunted since the early 1960's, they were still relatively shy of the boat. As we came upon a group of eight to ten whales in shallow water, they quickly scattered in all directions. We singled out and pursued one whale. In its fear the animal kept a very low profile, lifting only a small portion of its head above the water for rapid but infrequent breaths. This confounded our attempt to biopsy the animal. Eventually the whale did lift a portion of its back out of the water and we were able to get a shot just to the left of the dorsal ridge. However, we feel that this method of pursuit is not appropriate for belugas in the St. Lawrence Estuary. The pursuit of the whale causes more trauma than the biopsy itself.

Out of three hits on the lower back with an arrow fired from a crossbow, the whale only reacted noticeably to one hit. The whale reacted the most violently to a forceful downward thrust of the jab stick (it exploded with a thrash of its tail stock and slapped the water as it dove).

RECOMMENDATIONS FOR THE ST. LAWRENCE

Beluga whales in the St. Lawrence seem to be accustomed to marine traffic and are even known to approach boats, possibly out of curiosity (Robert Michaud, pers. comm.). This is an important attribute for researchers conducting behaviourial studies. Pursuing these whales may destroy this rapport as well as risking undue stress and exhaustion. We suggest instead that skin biopsies be collected opportunistically by field researchers whenever the whales approach their boat. One of two methods could be employed. A small, sharp handheld biopsy punch, similar to those used on human beings, could be used to quickly and lightly pull a small core of skin from the whale. A slap of the hand would create much less disturbance than the force of a jab stick. Another method would be to fire a biopsy dart from a crossbow using a relatively low draw weight. Although this may startle the whale at first, it will not disturb the other animals in the group. Provided that the boat remained stationary, the whales should continue their normal behaviour, which may help calm the startled whale.

The arrow could be retrieved in one of two ways. Brightly coloured arrows with floatation collars can be sighted and scooped from the water after the whales have left the area so that motoring over to the arrow does not disturb the animals. Alternatively, a fishing line could be attached to the arrow and played out by a spin casting reel affixed to the crossbow. Although this creates some drag on the arrow in flight, reeling in the arrow makes retrieval simple.

BIOPSY TIP DESIGN

The biopsy tips we used on the Winisk belugas have proved successful in collecting skin samples from right whales. However, they did not retrieve a sample from belugas. We could see clearly that the tip penetrated the whale's skin, but it rebounded without retaining any tissue. We suspect that the design of our biopsy tip is inappropriate for beluga skin. Possibly beluga skin is too thick or contains more connective tissue than the baleen whales, requiring a better system of barbs within the tip to retain the tissue. It is imperative that both a reliable biopsy tip for arrows and a handheld biopsy punch be designed and tested on a beluga carcass first before biopsy work can be conducted on belugas in the St. Lawrence.[1]

CONCLUSION

Based on our experience biopsying belugas in the Winisk River, we recommend that St. Lawrence belugas not be pursued. Rather, samples should be collected opportunistically by field workers, doing ongoing research, when belugas make a close enough approach. Biopsy punch and arrow tip design, as well as crossbow draw weight, must be perfected through tests on a beluga carcass first in order to insure that they will work reliably in the field.

[1] Further work by Nathalie Patenaude indicates that a variety of biopsy tip designs do retrieve tissue from beluga carcasses. Difficulty may be encountered with respect to the angle of hit, distance from target, draw weight of the crossbow, or water washing over the back during field tests with free swimming animals.

Figure A1. Winisk River Estuary and biosy equipment. **A:** Launch site at the Winisk River. A major difficulty we faced was that the river was shallow with many branching channels. We could only launch and return at high tide. Our Cree guide was very helpful in knowing where the deeper channels were and in keeping us from getting lost. Note the road at left leading to the airport, which permitted us to transport our gear from the plane to the water's edge. **B:** Crossbow and biopsy darts. An Astro Daco model 1000 crossbow with a 150 lb. draw weight was used to biopsy dart beluga whales. The crossbow bolts were modified with brightly coloured floatation collars and biopsy tips. **C:** Close up view of biopsy tip. Note the straightened fish hook barb inside for retaining the tissue sample, and the holes in the biopsy tip to allow air and water to escape upon impact.

A.

Ree Brennin

B.

Ree Brennin

C.