POPULATION GENETICS OF

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Assessment of inbreeding by DNA fingerprinting and assessment of biopsy darting factors for minimal wounding and effective sample retrieval

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ABSTRACT

The endangered St. Lawrence beluga (*Delphinapterus leucas*) population is not recovering from severe depletion despite its protected status over the past 20 years. DNA fingerprinting analysis of St. Lawrence beluga whales with three minisatellite probes (Jeffreys 33.6, 33.15 and M13) indicate a reduced level of genetic variability compared to Mackenzie Delta animals. The average band-sharing between individuals of the St. Lawrence beluga population for the three probes (0.534, 0.573, 0.478) was significantly higher than the average band-sharing of the Mackenzie Delta beluga population for the same probes (0.343, 0.424, 0.314). Higher levels of mean homozygosity in the isolated St.Lawrence belugas (0.33 vs 0.21) as well as a high degree of relatedness suggest that this population is inbred and that inbreeding depression is a factor in the lack of recovery of the St.Lawrence beluga population. Because sampling of some beluga populations may be biased, there is the need of alternative sampling procedures such as biopsy darting. To evaluate the impact of biopsy darting on beluga whales, different combinations of dart and stop sizes were tested on fresh beluga carcasses and the effect of different factors on the success of retrieval and the extent of wounding were evaluated. Tips with smaller diameters were more likely to retrieve a sample than those with larger diameters ($p < 0.05$) and longer tips were also more likely to retrieve a sample than shorter tips ($p < 0.10$). The force of impact, a function of draw weight and distance, had a significant effect

on the severity of wounding ($p < 0.05$). The samples obtained from all biopsy darts tested yielded sufficient amounts of DNA for genetic analysis.

PREFACE

The main objective of this study was to obtain a comparative measure of genetic variability for the St. Lawrence beluga whale population as compared to the Mackenzie Delta population. St. Lawrence beluga samples were collected by Pierre Béland of the Institut National d'Ecotoxicologie du St.-Laurent from dead beached whales. Samples for the Mackenzie Delta control population were obtained through native hunting. I extracted DNA from 20 St. Lawrence beluga samples (17 of which were of sufficient quality to obtain fingerprints) and 21 Mackenzie Delta samples and generated fingerprints from three different minisatellite probes. I have analysed the data, performed the statistical analysis when required, drafted the figures and tables and writen the manuscript. This work is presented in the first chapter in paper form for submission to the Canadian Journal of Zoology.

I also investigated the technique of biopsy darting as a method of collecting skin samples from free-ranging beluga whales. I spent a summer's field work at Shingle Point, Yukon Territory, to test this technique on beluga carcasses. I tested different dart sizes, crossbows and distances and developed an effective sampling method while inflicting minimal wound on the whale. I extracted DNA from various skin plugs, ran the statistical analysis to test various parameters, drafted the tables and figure and wrote the manuscript. This body of work is presented as the second chapter in paper form that will be submitted to Marine Mammal Science. The chapters have been formatted to comply with each journal's instruction to authors.

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"Dites, si seulement c'était vrai..."

Jacques Brel

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INTRODUCTION

Beluga whales (Delphinapterus leucas) in Canadian waters have been subdivided into six populations based upon geographic discontinuity or morphometric differences: Cumberland Sound, eastern Hudson Bay, western Hudson Bay, high Arctic, Beaufort Sea and the St. Lawrence (Sergeant and Brodie 1975). The latter is the southernmost population of this primarily arctic species. During the last century, this isolated population has been drastically reduced by commercial and bounty hunting (Pippard 1985), and was used in target practice during bombing exercises. As a result, the population was drastically reduced from more than 5,000 to a few hundred individuals (Sergeant and Brodie 1975,Pippard 1985; Sergeant 1986), and this has lead to its inclusion as an endangered population (COSEWIC 1988). Despite its protected status population surveys indicate this population is stable or decreasing.

A number of hypotheses have been offered to account for the failure of the St. Lawrence population to recover (Reeves and Mitchell 1984). One set of hypotheses supports extrinsic factors such as harassment, habitat degradation or contamination by toxic chemicals (Béland, Vézinat and Matineau 1988). Another set of hypotheses invokes intrinsic factors such as the genetic status of the population. The reduction in the number of individuals in a population leads to a loss of genetic diversity and increases inbreeding. Inbreeding can reduce the fertility of adults and increase juvenile mortality and may increase susceptibility to pathogens (Yuhki and O'Brien,

1990).

The objective of this study was to evaluate the degree of genetic variability found in the St. Lawrence beluga population by use of genetic fingerprinting. To date, most research has focused on the toxicity of the environment and its possible effects on the health and reproduction of the population (Muir 1990; Massé et al. 1990; Shugart 1990; De Guise et al. in prep.). The study presented in this thesis is the first to investigate the genetic status of the St. Lawrence beluga population. The degree of genetic variability was explored using DNA fingerprinting. This technique has been shown to be a powerful tool to assess the degree of relatedness between individuals (Jeffreys et al. 1985; Kuhlein et al. 1990) and provides a measure of genetic variation in a population (Hoelzel and Dover 1990; Reeve and al. 1990). For this study, the Mackenzie Delta (or Beaufort Sea) population was used as the reference population. Estimates for this population range from 10,000 to 15,000 individuals (Finley et al. 1987).

Obtaining tissue samples from whales in general is often limited and biased. In the specific case mentioned previously, samples were obtained from dead beached whales and may represent a bias if closely related individuals are more susceptible to lethal pathogens. Samples for the Mackenzie Delta control population were obtained through native hunts and may also be biased as hunted whales travelling in groups may represent family units. To alleviate these biases there is the need for an alternative sampling method. The technique of biopsy darting has been successfully used on a wide range of whale species (Brown et al. 1991; Matthews et al. 1988;

Lambertsen 1987; Weinrich et al. 1991; Whitehead et al. 1990; Arnason et al. 1985). This method consists of a projection unit (usually a crossbow) and a biopsy dart mounted on an arrow. The dart includes a hollow shaft with a collar at its base to prevent deeper penetration of the skin. The skin plug obtained is secured in the tip by a hook or a set of barbs.

There is no published data on biopsy darting of beluga whales. Furthermore, no study has documented the extent of wounding caused by biopsy darting. The objective of the second study presented here was to test various darts on beluga carcasses, document the effect of various parameters on wounding and therefore develop an effective biopsy darting method for collecting skin samples from free-ranging belugas.

(To be submitted)

Reduced genetic variability of the St. Lawrence beluga whale population as assessed by DNA fingerprinting

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PATENAUDE,N.J., QUINN, J.S., BELAND, P., and WHITE, B. N. Reduced genetic variability of the St. Lawrence beluga whale population as assessed by DNA fingerprinting.

Recent surveys suggest that the endangered St. Lawrence beluga (*Delphinapterus leucas*) population is not recovering despite being protected for the past 20 years. Dead individuals that have been autopsied show high levels of tumours and infections. This situation could be a result of pollution, inbreeding or a combination of both. Analysis of DNA fingerprints from St. Lawrence belugas with three minisatellite probes (Jeffreys 33.6, 33.15 and M13) indicate a reduced level of genetic variability when compared to Mackenzie Delta animals. The average band-sharing between individuals of the St. Lawrence beluga population for the three probes (0.534, 0.573, 0.478 respectively) was significantly higher than the average band-sharing of the Mackenzie Delta beluga population (0.343, 0.424, 0.314 respectively). Higher levels of mean homozygosity in the St.Lawrence belugas (0.33 vs 0.21) as well as a high degree of relatedness suggest that this population is inbred and that inbreeding depression could be a factor in the lack of recovery of the St.Lawrence beluga population.

Introduction

The global population of the beluga whale (*Delphinapterus leucas*), an essentially arctic species, numbers around 50,000 (Reeves 1990). Though this species has been accorded an "insufficiently-known" status by the International Union for Conservation of Nature and Natural Resources (IUCN 1988), some populations are known to be severely depleted. Of six beluga populations in Canadian waters (High Arctic, east and west Hudson Bay, Mackenzie Delta, Cumberland Sound and St. Lawrence), two are on the Committee on the Status of Endangered Wildlife in Canada listings as endangered (COSEWIC 1988). One of the endangered populations is found in the St. Lawrence Estuary (the other being in east Hudson Bay). This population has an annual distribution restricted to the St.Lawrence River and Estuary. Despite occasional sightings from the Canadian east coast and northeastern United States (Reeves and Katona 1980), this population is apparently isolated geographically and genetically from the northern populations (Pippard 1985) , the closest population being the Ungava Bay population, now believed to number very few individuals.

The St. Lawrence beluga was hunted heavily at the turn of the century and in the early and mid-nineteen hundreds (Pippard 1985). Additionally, belugas were thought to be responsible for cod and salmon stock depletion and so, were hunted for bounty. In the late 1920's and early 1930's St. Lawrence beluga whales were also used as targets for bombing exercises by the Canadian air force. It has been estimated that this

population numbered over 5 000 individuals in 1900 (Reeves and Mitchell 1984; Béland et al. 1988). By 1960, commercial whaling had ceased and the population numbered less than 1,200 (Pippard 1985). In 1979 St. Lawrence belugas were given protected status (Sergeant 1986). Since then several surveys indicate a population of between 350 and 500 animals that is either stable or decreasing in numbers (Reeves and Mitchell 1984; Pippard 1985; Sergeant 1986; Sergeant and Hoek 1990).

The St. Lawrence population has been studied extensively (Pippard 1985; Sergeant 1986; Béland et al. 1987; Martineau et al. 1987; Béland et al. 1988; Martineau et al. 1988; Caron 1990; Finley 1990; Sergeant and Hoek 1990). The lack of recovery despite protection has been attributed to many environmental factors including habitat degradation, harassment by boating and toxic contamination (Reeves and Mitchell 1984). Most research has focused on the toxicity of the environment and its possible effects on the health and reproduction of the population (Muir 1990; Massé et al. 1990; Shugart 1990; De Guise et al. submitted).

Genetic profiles have been generated using variable number of tandem repeat (VNTR) markers to study several mammalian populations (Jeffreys et al. 1985b; Jeffreys et Morton 1987; Hoelzel and Dover 1991; Wayne et al. 1991; Amos et al. 1991). This technique examines many loci at once by exploiting the presence of regions of DNA that contain multiple repeats of a minisatellite sequence (Jeffreys et al. 1985a). It is thought that most of these loci are unlinked, segregate independently in pedigrees (Jeffreys et al.1985b; Jeffreys et al. 1986; Burke and Bruford 1987) such that on average, an individual receives half of its DNA fingerprint bands from each parent. Profiles

generated by DNA fingerprinting (Jeffreys et al. 1985a) can be used to assess the degree of relatedness between individuals (Kuhlein et al. 1990; Jones et al. 1991; Brock and White 1992). By scoring the number of bands shared between presumably unrelated individuals in a population such as the St. Lawrence beluga we can obtain a relative measure of genetic variation as compared to other populations. When a population becomes chronically inbred by either generations of consanguineous mating or because of prolonged population bottlenecks then VNTR alleles may become fixed at some loci, giving rise to a higher mean band-sharing among individuals. DNA fingerprints are thus more similar between individuals from an inbred population than those from an outbred population (Kuhlein et al. 1990; Bellamy et al. 1991).

Our study addresses the genetic status of the St. Lawrence beluga population. The decline in population size leads to a reduction of genetic diversity and increases the potential of inbreeding. Though low levels of genetic variation do not necessarily lead to inbreeding depression (Hartl and Clark 1989), recent studies have shown an inverse correlation between levels of inbreeding between mated pairs and the probability of breeding success and juvenile survival rates (Ballou and Ralls 1982; Ralls et al. 1988; De Bois et al. 1990; Brock and White 1992). Mortality was found to be 33% higher on average in 38 species of mammals for progeny of first degree matings then for progeny of unrelated parents.

Inbreeding depression is generally believed to result from increased homozygosity or lowered heterozygosity (Charlesworth and Charlesworth 1987). The fitness of individuals is decreased under conditions of inbreeding because of the increased

expression of low frequency deleterious genes that are normally masked by dominant alleles under conditions of outbreeding. Inbreeding also lowers the overall proportion of heterozygous loci and for some loci it has been suggested that heterozygosity could be more advantageous to the individual than even the most favourable homozygous combinations (overdominant hypothesis) (Allendorf and Leary 1985; Ziehe and Roberds 1989). Inbreeding depression may be one of the elements involved in the St.Lawrence beluga population's failure to recover. Population surveys suggest that the St. Lawrence beluga's reproductive rate is half or less than half than that estimated for Arctic animals (Sergeant and Hoek 1990). Béland et al. (1988) suggested that the borderline between increasing and decreasing beluga populations was defined by populations showing 28 to 30% of grey animals. The percent of greys in the St. Lawrence population was estimated between 21 and 26% of the total population (Sergent 1986; Béland et al. 1987) suggesting that the ratio of young to adult is below what is required for the population to expand.

The goal of this our study was to examine the level of genetic variation found in the St. Lawrence beluga population by use of DNA fingerprinting. In this study, we have compared the level of genetic variability of the St. Lawrence beluga population to that of the Mackenzie Delta. The number of individuals that summer in the Mackenzie Delta is estimated at over 11,500 individuals (Fraker 1980; Finley et al. 1987) and though they are hunted by Inuits there is no indication of population instability or decline (Norton-Fraker and Fraker 1982).

Material and methods

Sample collection

Beluga skin samples were collected from Inuvialuit kills in the Mackenzie Delta, Beaufort Sea (68.50/136.25) and from dead beached animals in the St. Lawrence Estuary (48.26/68.33). Samples were obtained through the cooperation of the Fisheries Joint Management Committee and L'Institut National d'Ecotoxicologie du St.-Laurent. Skin samples were shipped frozen or in a NaCl saturated solution (0.25 M EDTA, pH 8.0; 20% DMSO) and stored at -20^{0}C upon arrival.

DNA isolation

Skin samples (0.50 - 0.75 g) were ground to a fine powder in liquid nitrogen and suspended in 3.5 ml lysis buffer (4 M urea, 0.2 M NaCl, 0.1 M. Tris HCl,pH 8.0, 0.5% n-laurylsarcosine, 10 mM EDTA), followed by a proteinase-K(72 units) digestion from one to three weeks at 37^{0}C. DNA was extracted twice with phenol and chloroform (70:30) and once with chloroform. The DNA was then precipitated by adding sodium acetate to a final concentration of 0.15 M and two volumes of 95% cold ethanol. The DNA pellet was washed with 70% ethanol, air dried and dissolved in 0.5 ml TNE_2 (10 mM Tris-HCl, 10 mM NaCl, 2 mM EDTA, pH 8.0) overnight at 37^{0}C.

DNA fingerprints

DNA fingerprints were obtained using techniques similar to those described in Brock and White (1991). Genomic DNA (5*ug*) was cleaved with the restriction enzyme *Hae*III (3 units/*ug* of DNA) according to the manufacturer's directions (Bethesda Research Laboratories). We added a lambda DNA digest to each sample as a size marker and as an internal control for differential mobility (Brock and White 1991; Galbraith et al. 1991) Each sample was loaded on a 0.8%, 20 cm agarose gel and electrophoresis was carried out at 1.5 volts per cm for 48 h. in recirculating buffer (45 mM Tris-borate pH 8.0, 1 mM EDTA). The DNA was transferred to a positively charged nylon membrane (Immobilon-N, Millipore Corporation) by capillary flow following the technique of Southern (1975).

The blots were prehybridized overnight at 65^{0}C in a solution of 7% SDS, 1 mM EDTA (pH 8.0), 1% bovine serum albumin, 0.25 M sodium ortho-phosphate (pH 7.2) (Weastneat et al. 1988). Probes were radioactively labelled by primer extension (Feinberg and Vogelstein 1983) with [alpha^{32}P]dCTP. Blots were hybridized overnight at 65^{0}C with Jeffreys's 33.6 or 33.15 minisatellite probes (33.15 was cloned into M13) (Jeffreys et al. 1985b). Blots probed with Jeffreys 33.6 or 33.15 were washed once in 2 X SSC, 0.1 % SDS at room temperature for 15 minutes, then once at 65^{0}C for 15 minutes in 2 X SSC, 0.1% SDS, once at 65^{0}C for 30 minutes with the same solution. Prehybridization and hybridization with M13 minisatellite probe were carried out at 60^{0}C (Westneat et al. 1988). Blots probed with M13 were washed in a 2 X SSC, 0.1% SDS twice at room temperature and once at 60^{0}C (Westneat et al. 1988). Blots were exposed

to X-Ray film (Dupont Cronex or Kodak) at -70°C for 1-15 days with one intensifying screen, then stripped in 0.4 N NaOH at room temperature for 30 min prior to reprobing. Blots were finally rehybridized with [alpha^{32P}]dCTP-labelled lambda DNA under the same conditions described above for the Jeffreys 33.6 and 33.15 minisatellite probes.

DNA Fingerprint analysis

Fingerprints were scored from the first detectable band in the most sheared sample to the point at which crowding of the low molecular weight bands prevented scoring. The molecular weight range scored varied for each probe. Fragments which had a co-migrating counterpart of roughly similar autoradiographic intensity where scored as identical bands. Fragments that were less than half the intensity of their co-migrating counterparts were disregarded because in such cases it was not possible to determine if the lesser intensity band was also present in the higher intensity band. The DNA fragments detected by each probe were identified by measuring the distance migrated to the closest internal lambda size marker (Galbraith et al. 1991). A band was considered identical in two individuals if the migration distance was within 0.5mm of each other. This was found to be the maximum difference in the measurement of mobility of bands for triplicate samples placed at outer lanes and in the center lane of the gels.

DNA fingerprint band-sharing coefficients, D, were calculated as

$$D = \frac{2*Nab}{Na+Nb}$$

where Nab is the number of shared bands between two individuals and Na and Nb are the total number of bands scored in each individuals (Wetton et al. 1987). Average band sharing coefficients were calculated by taking the overall mean of the mean D for each individual to minimize lack of independence in the data set.

The coefficient of relatedness 'r' was calculated as a measure of the effect of inbreeding with

$$r = \frac{D_1 - D_2}{1-D_2}$$

where D_1 is the expected band-sharing coefficient of the St. Lawrence beluga population and D_2 is the band-sharing coefficient of the Mackenzie Delta population (Lynch 1991).

The probability (x) that an allele in individual A is also present in individual B was calculated by

$$x = \frac{Nab/Na + Nab/Nb}{2}$$

If co-migrating bands in two individuals are always identical alleles of the same mini-satellite locus, then the mean probability ($\bar{x}$) is related to the frequency 'q' of that allele by

$$\bar{x} = 2q-q^2$$

Assuming there is little variance in frequency between alleles, the mean allele frequency '$\bar{q}$' can be calculated for each population by

$$\bar{q} = 1- (1-\bar{x})^{0.5}$$

The number of alleles approximating

$$n = 1/q$$

then the mean homozygosity 'H_o' can be approximated by

$$\Sigma q^2 = n\bar{q}^2 = \bar{q} = H_o$$

(Crow and Kimura 1970; Jeffreys et al. 1985b).

The hypothesis that the average observed D for each probe (M13, 33.6 and 33.15) in the St. Lawrence population were greater than that observed for the Mackenzie Delta population was tested with two-sample t-tests.

Results

The three probes M13, Jeffreys 33.6 and 33.15 were used to generate DNA fingerprints for 17 St. Lawrence belugas and 20 (21 for 33.6) Mackenzie Delta belugas (Figs. 1, 2 and 3). Previous screening with Per and 3'HVR minisatellite probes showed weak hybridization to beluga DNA. Screening of DNA digested with the restriction enzymes *EcoR*I, *Mbo*I, *Rsa*I, *Hinf*III and *Hae*III showed *Hae*III gave the best scorable DNA fingerprints. There was some overlap of bands identified by probes 33.15 and M13 due to the fact that 33.15 was cloned into M13 (proportion of fragments scored with M13 that were detected by 33.15 was 0.52 +-0.024 (SD) for St. Lawrence and 0.69 +-0.041 (SD) for Mackenzie Delta). This overlap is higher than that observed by Westneat (1990) (overlap=0.26). The mean number of scored bands (Table 1) for each probe is comparable to other studies of similar size ranges for 33.6 and 33.15 (Jeffreys et al. 1985b, Wetton et al. 1987). M13 detected on average more bands than 33.6 or 33.15 and more bands than for similar kb ranges in other species (Westneat et al. 1990). This may be due to the less stringent washing protocol for M13 probing.

The sample size for this study was limited by two factors. Firstly, the St. Lawrence beluga is a protected population so the only samples available were obtained from the limited number of recovered beached carcasses. Secondly, because the St. Lawrence beluga carcasses were sometimes recovered several days after beaching, the DNA was degraded and this limited its use in fingerprinting analysis (DLE 186, DLE

336 and DLE 340 in Fig 1,2 and 3).

The number of bands scored in the DNA fingerprint per individual and the D values of presumably unrelated individuals from the Mackenzie Delta population and from the St. Lawrence beluga population for each probe are reported in Appendix A. The frequency distributions of D values for each probe and the combined average showed higher levels for the St. Lawrence beluga population (Fig.4). These differences in distributions were the first evidence suggesting that the St. Lawrence beluga population are more related to each other than the Mackenzie Delta animals are to each other.

The mean band-sharing coefficients were consistently higher for the St. Lawrence than the Mackenzie Delta belugas (Table 1). For each probe, the mean D of the St.Lawrence beluga population was significantly higher ($p < 0.0001$) than that of the Mackenzie Delta belugas. To approach the question of how related St. Lawrence belugas might be to each other, we calculated the coefficient of relatedness for each probe. Assuming the D for unrelated Mackenzie Delta individuals represent bands that are identical in state (or background band-sharing coefficient)(Lynch 1990), the mean estimated coefficient of relatedness within the St.Lawrence samples across three probes was 0.28, SEM =0.012 (Table 1).

Mean homozygosity and mean allele frequency for the St. Lawrence beluga population was more than 50% higher than that of the Mackenzie Delta population (Table 1). The average mean homozygosity for the three probes for the St.Lawrence beluga population was 0.33 while the average mean homozygosity for the Mackenzie Delta was 0.21. These estimates of mean allele frequency and homozygosity are maximal because

an unknown proportion of co-migrating bands in two individuals will be obtained by chance (Jeffreys et al. 1985b). Mean homozygosity calculations using a formula suggested by Lynch (1990) gave similar results.

Discussion

We have evaluated the level of genetic similarity among individuals in the St. Lawrence beluga whale population by use of DNA fingerprinting. In general, genetic similarity has been shown to be relatively low in outbred populations while studies on known inbred and domestic populations showed higher levels of genetic similarity (Georges et al. 1988; Kuhlein et al. 1990). Some natural populations such as the Channel Island fox and the naked mole rat show extreme levels of genetic similarity (Gilbert et al. 1990; Reeve et al. 1990). These observations may be explained by recent common ancestry or founder effect and may be maintained by ecological constraints. Our study shows that while fingerprints generated from probes M13, 33.15 and 33.6 gave three different D scores for each beluga population, in each case the mean D score was significantly higher for the St. Lawrence population, indicating lower levels of genetic varibaility than for the Mackenzie Delta population.

Our results may be biased with respect to the degree of relatedness of the St. Lawrence beluga sampled because closely related individuals may share a higher susceptibility to pathogens. Samples were obtained from dead beached animals and the causes of deaths have not been established though these animals were found with severe pathologies (De Guise et al. in prep). One way to overcome this potential sampling bias is to sample free-ranging beluga whales by biopsy darting. This technique has been successfully used on several whale species to retrieve small skin samples for genetic

analysis (Winn et al. 1973; Lambertsen 1987; Matthews et al. 1988; Whitehead et al. 1990; Brown et al. 1991; Weinrich et al. 1991) and is being assessed for beluga whales (Patenaude and White, in prep).

Expected levels of inbreeding are usually based on calculations that involve population size (Crow et al. 1970), but in this study there was not enough adequate demographic data for direct calculations of inbreeding. Population surveys for belugas and for cetaceans in general are difficult to conduct, sexing of beluga whales is often inaccurate and the overall data of the St. Lawrence population regarding age structuring and male/female ratio are incomplete (Reeves and Mitchell 1984; Béland et al. 1987; Kingsley 1990). Therefore we used band-sharing as an indirect measure of inbreeding by assuming that D scores of Mackenzie Delta animals were representative of the St. Lawrence beluga population prior to bottleneck. It may be argued that the Mackenzie Delta population numbers three times more than the St. Lawrence population did in early 1900's and therefore would be expected to hold increased genetic diversity. The estimates for the St. Lawrence belugas population size in 1900 was based on an 8% growth rate. If in fact the population's growth rate was in fact 4%, then the initial population size was in fact 10,000 (Béland 1990). More so, belugas from eastern and western Hudson Bay and Ungava Bay are thought to overwinter together in Hudson Strait (Finley et al. 1982). Mating is thought to occur in spring (Sergent 1973) while many belugas are still in wintering areas. Some belugas winter along the coast of Labrador and before the depletion of both the eastern Hudson Bay and the Ungava Bay beluga populations in the late 1800' (Finley et al. 1982), there was most likely some gene flow between the St.

Lawrence and eastern Arctic populations (Richard and Orr 1990). The reduction in numbers by these populations and the St. Lawrence population may have prevented further genetic exchange.

The factors that may affect band sharing coefficients of the control population are twofold. First, samples were obtained through native hunts and the hunted whales may travel in groups representing family units. Thus the D values between the sampled whales could be an overestimate of the population at large. Secondly, the samples were obtained from five different hunting camps in the Mackenzie Delta estuary (Shingle Point, Kendall Island, East Whitefish station, West Whitefish station, Tuktuyuktuk and Hendrickson Island) (Appendix B). If subpopulationing between east and west Mackenzie Delta summering sites is observable within the nuclear genome then overall band-sharing estimates would lower the level of genetic similarity actually present in the Mackenzie Delta population. We have tested for difference of D values within the East and West populations as compared to the overall mean and found no significant difference for five of the six subpopulation/enzyme combinations (t values ranging between -1.21 and 0.42). The sixth combination of West subpopulation/Jeffreys 33.15 gave a lower index of similarity than the overall mean for the total population ($D = 0.272$, SEM 0.09; $t = -2.53$, $p < 0.01$).

Band-sharing coefficients are linearly related to the degree of relatedness (Kuhlein et al. 1990; Lynch 1991; Brock and White 1992). In principle the band-sharing coefficient (D) provides a nearly unbiased estimate of relatedness (r) when the background level of genetic similarity is very low. As the average similarity between

non relatives increases D becomes a less sensitive indicator of r because band-sharing distributions overlap for different levels of kinship (Lynch 1990; Capy and Brookfield 1991; Piper and Rabenold, in press). Nevertheless, it is generally agreed that D provides an adequate estimate of average amount of kinship for a population (Lynch 1990; Capy and Brookfield 1991). The relative mean 'r' value of 0.28 estimated for the St. Lawrence population corresponds to that of second degree relatives and suggests a high level of inbreeding. Some authors have cautioned interpretation of VNTR data (Lander 1989; Cohen 1990; Lynch 1990). In some cases, DNA-fingerprint bands cannot be treated as independent estimators of relatedness because of possible linkage (Brock and White 1991). The three different probes gave the same relative level of genetic similarity, suggesting that there is little or no linkage disequilibrium.

If the St. Lawrence belugas that were sampled represent the genetic status of the population then inbreeding may in part explain the lack of recovery. Mean levels of homozygosity for the St. Lawrence population are fifty percent higher than those of the Mackenzie Delta population. Similarly , the mean allelic frequency is higher for the St. Lawrence population, signifying a reduction in the number of alleles present in the population. The maintenance of allelic diversity has important fitness benefits for a population experiencing changing conditions and for example reduced allelic diversity at immune system coding genes appears to be a cause of lowered disease resistance (May 1988, O'Brien and Evermann 1988). Low levels of heterozygosity at some genetic loci such as the major histocompatibility complex (MHC) may have direct consequences on the health of the population. Because the MHC is the most variable genetic complex in

terrestrial mammals and is thought to be pathogen driven (Klein 1986; Klein and Takahata 1990), reduced variability at these gene loci might increase risks and effects of diseases. Studies of St. Lawrence beluga carcasses have revealed severe pathologies including adenocarcinomas, broncho-pneumonias, testicular necrosis, chronic mastitis, stomach ulcers and others (De Guise et al. in prep.) which may be suggestive of a depressed immune system. We have begun an investigation of the genetic variability at the class II MHC loci in St. Lawrence beluga whales and results using human probes have shown limited polymorphism. However, these results are preliminary and other studies suggest that marine mammals may have limited variability at these loci in general (Trowsdale et al. 1989; Slade 1992).

Conservation and recovery plans should incorporate demographics and population genetics in assessing requirements for populations survival (Lande 1988). Very low levels of genetic exchange (roughly one individual per generation) would be sufficient to increase genetic variability in the absence of strong selection (Crow and Kimura 1970). One possibility would be to relocate a few Arctic beluga whales into the St. Lawrence Estuary. The choice of recruited whale should rest on an individual that is closely related in genetic structure to St. Lawrence animals as outbreeding depression remains a concern for overall population fitness (Templeton 1986). Outbreeding depression refers to the intrinsic coadaptation of gene complexes that evolve under the influence of other genes to the extent of incompatibility between divergent populations. MtDNA evidence suggests that the St. Lawrence and East Hudson Bay beluga populations are not genetically differentiated. These two population are thought to have

separated only 7,000 years ago (Brennin et al., in prep.) and present no real concern of outbreeding depression. Unfortunately, the eastern Hudson Bay population is also endangered and limited in numbers (population estimates are about 500).

Environmental factors such as habitat degradation, pollution, harassment and toxic contamination (Pippard 1985; Finley 1990; Massé et al. 1990; Muir 1990) have been suggested to be responsible for the St. Lawrence belugas lack of recovery. We suggest that inbreeding may also contribute to this lack of recovery. Our results suggest a genetic profile for the St. Lawrence population that is consistent with the inbreeding hypothesis. In view of the potential risks of inbreeding depression, future management decisions should consider increasing the genetic variability of the St. Lawrence beluga population.

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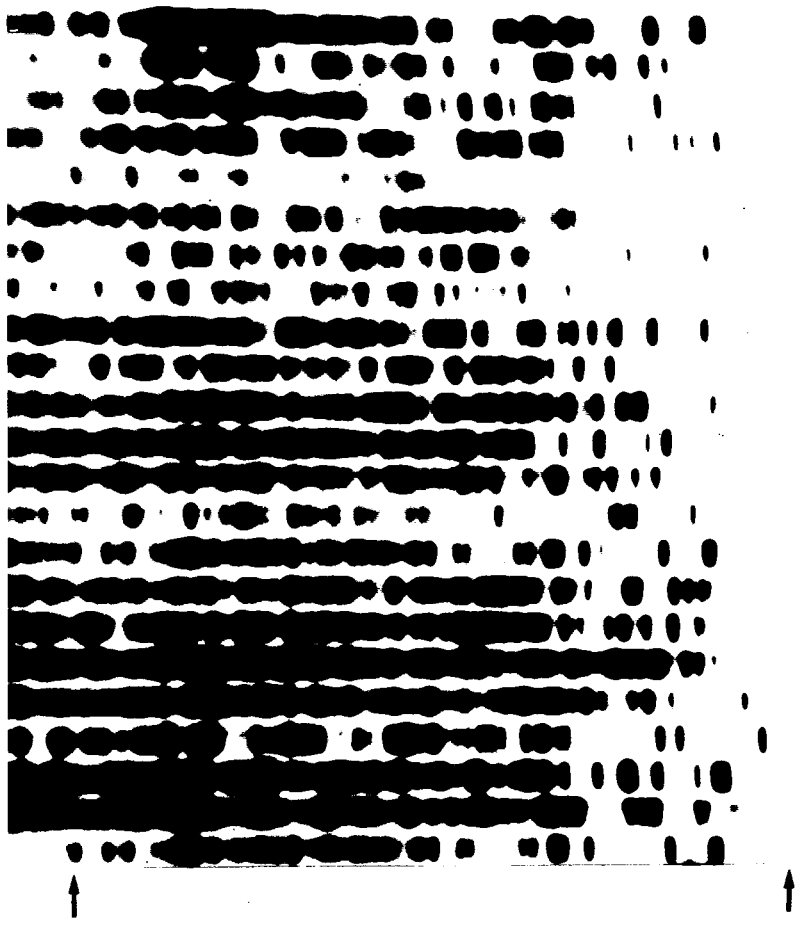
FIGURE 1. Multilocus DNA fingerprints of genomic DNA from a) St. Lawrence belugas and b) Mackenzie Delta belugas, digested with *Hae*III and probed with Jeffreys 33.15.

Animals were designated by their species name (Delphinapterus leucas) and by chronological order of their receipt.

a Lane not scored because of poor quality of fingerprint

→ Range in kilobases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DLE 281
DLE 241
DLE 243
DLE 268[a]
DLE 260
DLE 298
DLE 285
DLE 272
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DLE 88
DLE 89
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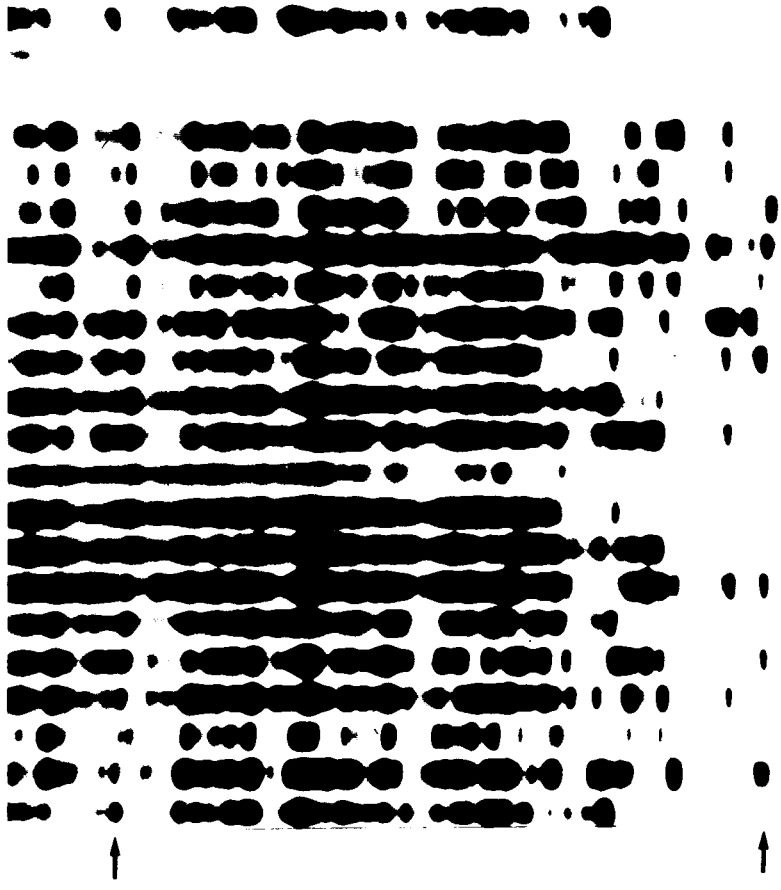
DLE 92
DLE 81
DLE 91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DLE 340[a]
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DLE 190
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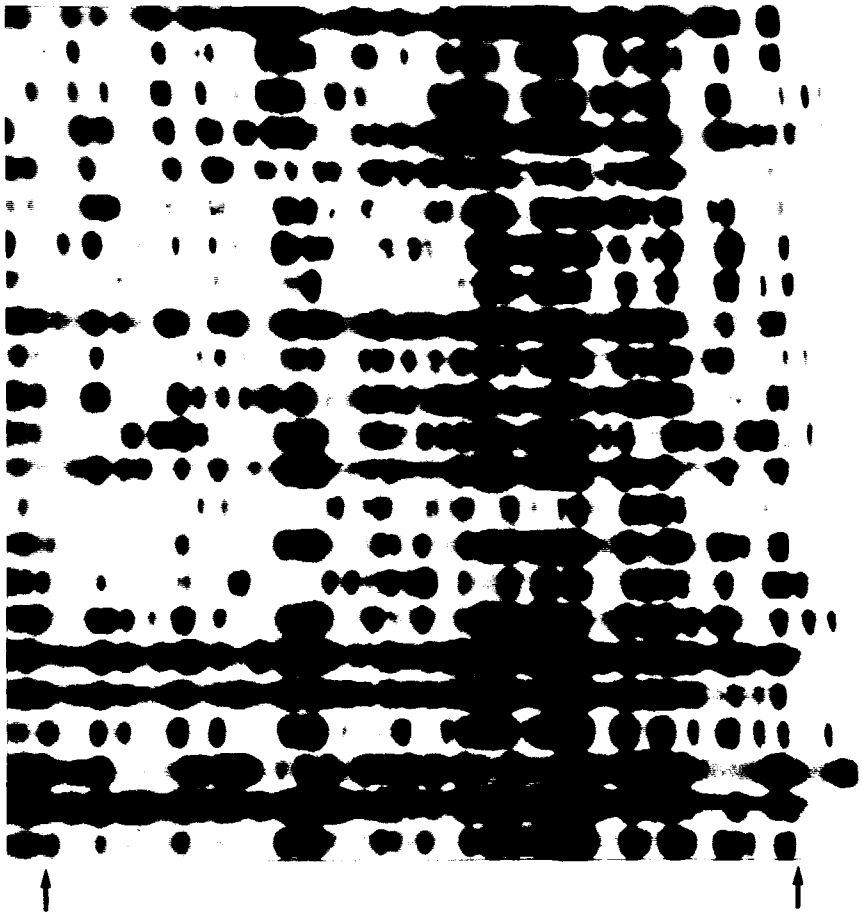
DLE 341
DLE 336[a]
DLE 334
DLE 239
DLE 192
DLE 342
DLE 189
DLE 188
DLE 187
DLE 185
DLE 371

FIGURE 2. Multilocus DNA fingerprints of genomic DNA from a) St. Lawrence belugas and b) Mackenzie Delta belugas, digested with *Hae*III and probed with Jeffreys 33.6.

a Lane not scored because of poor quality of fingerprint

→ Range in kilobases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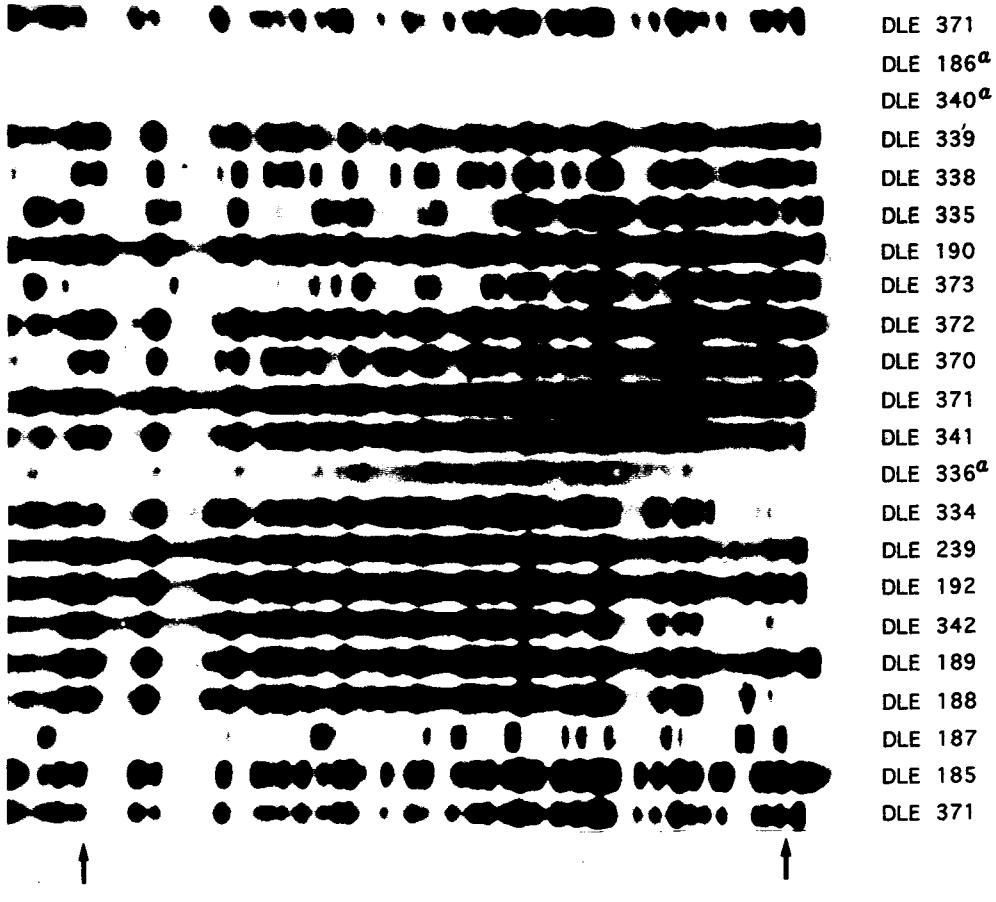
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DLE 336[a]
DLE 334
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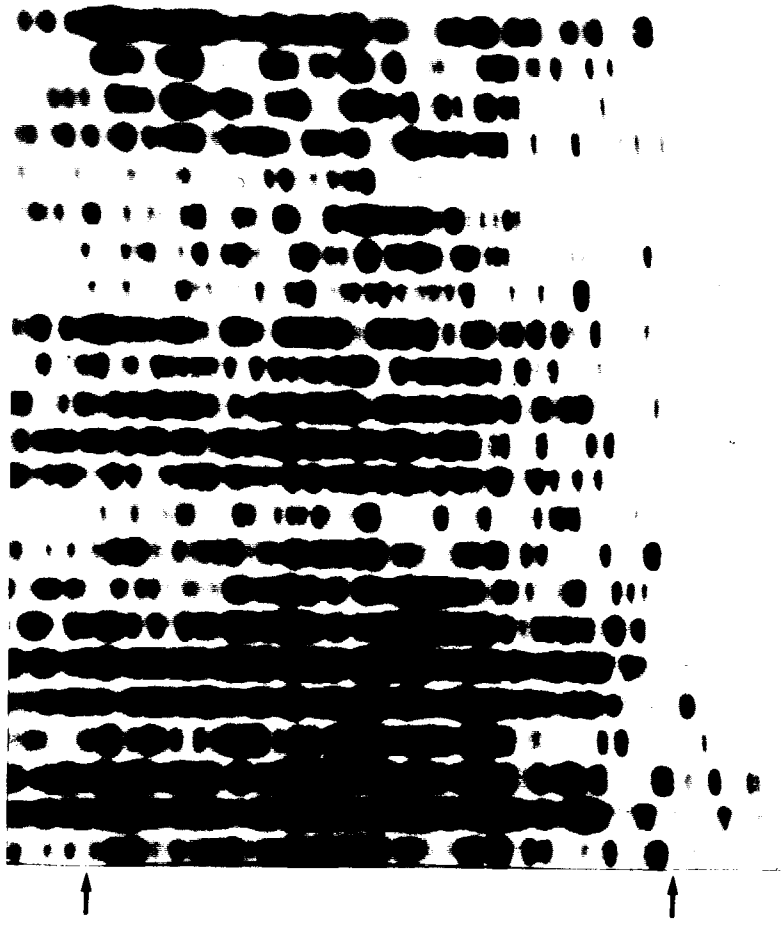
DLE 371

FIGURE 3. Multilocus DNA fingerprints of genomic DNA from a) St.Lawrence belugas and b) Mackenzie Delta belugas, digested with *Hae*III and probed with M13.

a Lane not scored because of poor quality of fingerprint

→ Range in kilobases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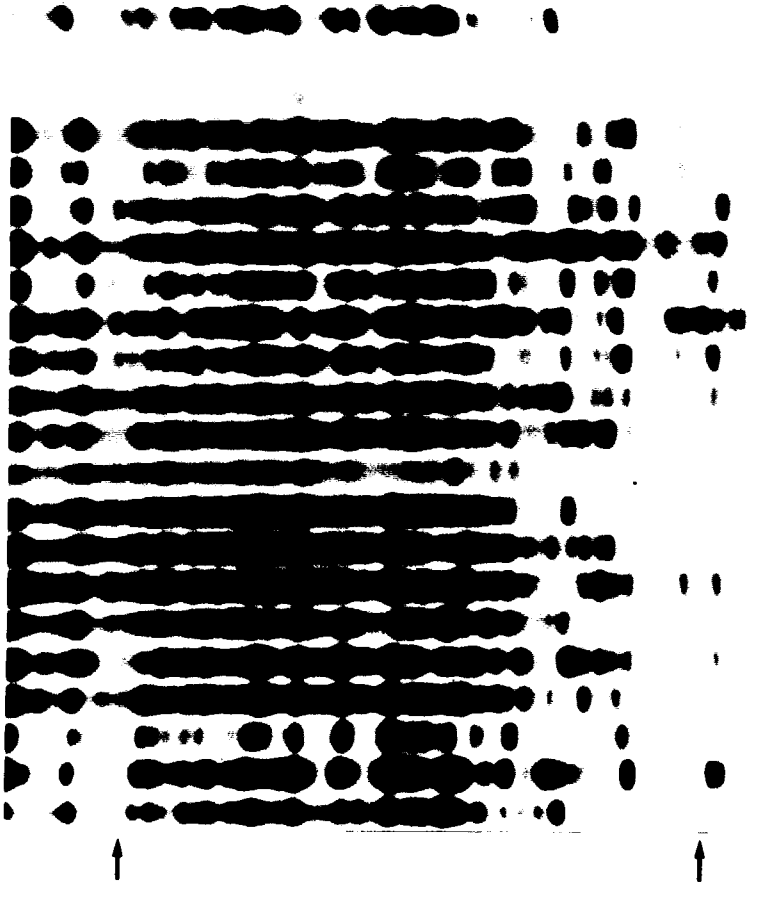
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FIGURE 4. Frequency distributions of mean fingerprint band-sharing probabilities from Mackenzie Delta belugas (□) and St. Lawrence belugas (■) using probes a) 33.15 b) 33.6 c) M13 and d) combined average over all three probes. The mean band-sharing coefficients for each individual were grouped into 5% intervals.

TABLE 1. Comparison of analysis of DNA fingerprints from unrelated Mackenzie Delta belugas and unrelated St. Lawrence belugas.

Probe	33.15	33.6	M13	Average
Size range (kb)	2.5-9.8	2.1-13.5	2.3-11.6	-
MACKENZIE DELTA				
Band-sharing coefficient (D) ± SEM	0.343 ±0.009	0.424 ±0.015	0.314 ±0.006	0.361 ±0.027
Mean no. bands (n) ±SD	19.1 ±3.7	17.7 ±4.2	28.7 ±3.1	
Band sharing probability (x) ± SEM	0.363 ±0.007	0.438 ±0.009	0.323±0.006	
Mean allele frequency (q)	0.202	0.250	0.177	0.21
ST. LAWRENCE				
Band-sharing coefficient (D) ± SEM	0.534 ±0.012	0.573 ±0.027	0.478 ±0.010	0.528 ±0.023
Mean no. bands (n) ± SD	14.3 ±2.4	22.4 ±4.8	24.4 ±2.5	
Band sharing comparison (x) ± SEM	0.550 ±0.010	0.596 ±0.016	0.505 ±0.007	
Mean allele frequency (q)	0.329	0.364	0.296	0.33
Degree of relatedness (r)	0.29	0.26	0.24	

(For M13: $t_{[35]}=15.4$, $p<0.0001$; For 33.15: $t_{[35]}=13.3$, $p<0.0001$; For 33.6: $t_{[36]}=5.2$, $p<0.0001$)
SD, standard deviation of the mean; SEM, standard error of the mean

APPENDIX A.

Tables A1 to A6. Number of bands scored in the DNA fingerprint per individual (diagonal) and band-sharing coefficients (D) between pairwise comparisons of individuals from the Mackenzie Delta beluga population and from the St. Lawrence beluga population for each probe.

Table A1. D scores between MacKenzie Delta beluga DNAs probed with M13.

	DL091	DL081	DL267	DL092	DL089	DL088	DL243	DL240	DL255	DL257
DL091	28	.2295	.3103	.3214	.4706	.4516	.2759	.4308	.3846	.3000
DL081		33	.3175	.3279	.3214	.3284	.3492	.4000	.1754	.3385
DL267			30	.3448	.3019	.4063	.4000	.3582	.3704	.2903
DL092				28	.2745	.3226	.3448	.4615	.3846	.4000
DL089					23	.3509	.4063	.4333	.3830	.2546
DL088						34	.3226	.5352	.4138	.4849
DL243							37	.4242	.3019	.2581
DL240								28	.2623	.3768
DL255									24	.3214
DL257										32

	DL263	DL271	DL272	DL285	DL298	DL260	DL248	DL241	DL281	DL251
DL091	.3214	.3103	.2857	.2546	.2546	.3077	.4286	.4561	.2308	.3158
DL081	.3607	.3175	.1967	.1667	.3333	.1404	.2951	.3871	.2456	.3871
DL267	.2414	.3000	.3448	.3158	.3860	.4074	.1724	.3729	.2593	.3390
DL092	.1786	.3448	.3214	.2182	.2546	.2692	.4286	.2807	.2308	.3860
DL089	.3529	.3020	.2353	.1600	.2800	.3830	.4314	.4615	.2128	.4231
DL088	.2581	.3750	.3226	.3279	.2623	.3448	.3871	.4762	.3793	.4127
DL243	.3793	.3333	.2414	.1788	.3214	.1509	.3509	.2264	.2963	.3729
DL240	.4308	.4478	.2462	.2500	.3438	.2951	.4615	.2951	.2951	.3939
DL255	.2692	.2593	.2692	.3137	.2353	.2917	.3846	.3019	.2917	.3396
DL257	.4000	.2581	.2333	.3390	.3390	.2500	.4000	.3607	.2143	.3279
DL263	28	.2759	.2500	.2909	.3273	.1923	.2500	.4211	.2692	.2807
DL271		30	.2759	.2807	.2456	.3704	.2759	.3051	.3333	.3390
DL272			28	.2909	.3636	.3846	.2500	.2807	.2692	.2807
DL285				27	.3333	.3137	.2909	.2745	.2857	.1786
DL298					27	.3529	.4000	.1961	.1961	.2857
DL260						24	.3077	.3333	.3333	.2264
DL248							30	.1539	.1539	.4211
DL241								29	.2264	.3793
DL281									24	.3396
DL251										29

N = 20
Mean D = 0.314 (s.e.m. = 0.006)
Mean number of bands scored = 28.7 (s.d. = 3.1)

Table A2. D scores between St. Lawrence beluga DNAs probed with M13.

	DL339	DL185	DL187	DL188	DL189	DL342	DL192	DL239	DL334	DL341
DL339	27	.6415	.5000	.5769	.6154	.3636	.5306	.4898	.4400	.5833
DL185		27	.5283	.4528	.4906	.3556	.4400	.6000	.5882	.5306
DL187			26	.6154	.6923	.4091	.5306	.5714	.6000	.5833
DL188				26	.6539	.5000	.5306	.4490	.4000	.5417
DL189					26	.5000	.5306	.5306	.5600	.6250
DL342						18	.4390	.2927	.5714	.4000
DL192							23	.3913	.3830	.4889
DL239								23	.5106	.5333
DL334									24	.5217
DL341										22

	DL370	DL372	DL373	DL190	DL335	DL338	DL371
DL339	.2176	.4727	.5000	.5091	.4082	.3846	.5714
DL185	.3830	.4643	.4528	.4815	.4167	.3774	.5200
DL187	.3913	.4364	.5000	.7170	.5106	.4615	.5306
DL188	.4783	.5818	.5769	.5283	.4681	.5385	.6531
DL189	.5652	.5091	.5385	.7170	.4681	.5769	.6122
DL342	.4737	.4681	.5000	.4444	.4103	.4091	.3902
DL192	.5116	.6154	.5306	.4800	.5000	.4898	.3478
DL239	.2791	.3846	.4898	.5200	.3636	.4082	.4783
DL334	.4546	.4906	.4800	.5882	.5333	.3600	.4681
DL341	.5714	.3922	.5000	.5714	.4186	.5000	.4889
DL370	20	.5306	.5217	.5106	.3902	.4783	.4651
DL372		29	.5455	.4286	.5600	.6182	.4231
DL373			26	.5283	.5532	.6154	.5714
DL190				27	.5000	.5283	.5600
DL335					21	.5106	.4546
DL338						26	.4082
DL371							23

N = 17
Mean D = 0.478 (s.e.m. = 0.010)
Mean number of bands scored = 24.4 (s.d. = 2.5)

Table A3. D scores between Mackenzie Delta beluga DNAs probed with 33.15.

	DL260	DL243	DL241	DL281	DL088	DL240	DL248	DL251	DL255	DL257
DL260	12	.5625	.4828	.4286	.4706	.3415	.1818	.2000	.4118	.5581
DL243		20	.4865	.3333	.5238	.4490	.1951	.4211	.4762	.4375
DL241			17	.4849	.4103	.3913	.3684	.2857	.3590	.4000
DL281				16	.3158	.3556	.4324	.1177	.3684	.3784
DL088					22	.2745	.4651	.4500	.4546	.3889
DL240						29	.3600	.3404	.5098	.4490
DL248							21	.2564	.3721	.3902
DL251								18	.5000	.2632
DL255									22	.3810
DL257										20

	DL263	DL271	DL272	DL285	DL298	DL091	DL081	DL267	DL092	DL089
DL260	.2286	.2143	.3125	.2857	.4444	.3333	.2941	.3030	.1212	.2500
DL243	.4651	.3889	.3000	.5000	.4571	.2632	.2857	.3902	.2439	.2500
DL241	.3500	.2424	.2162	.3636	.7500	.2857	.1538	.5263	.3684	.2759
DL281	.2564	.1875	.3333	.3750	.5161	.4118	.2632	.2703	.1622	.4286
DL088	.4000	.4211	.5238	.3158	.3243	.3500	.3636	.2791	.2791	.3529
DL240	.4231	.3556	.4898	.4000	.3182	.2979	.2745	.5200	.4000	.2927
DL248	.4091	.2162	.2927	.3243	.4444	.2051	.3256	.3333	.2857	.3030
DL251	.2927	.1765	.4737	.2941	.2424	.2222	.3500	.4615	.4103	.2667
DL255	.4000	.3158	.4286	.4211	.3243	.4500	.3182	.4651	.3256	.2353
DL257	.5581	.4444	.5000	.3333	.2857	.4737	.3810	.1951	.3415	.2500
DL263	23	.3077	.3256	.4615	.2105	.2927	.4000	.4546	.4091	.2857
DL271		16	.2222	.3125	.1936	.2941	.2632	.1622	.3243	.2857
DL272			20	.1667	.1714	.3684	.3810	.3415	.2927	.1875
DL285				16	.4516	.1177	.3684	.3784	.3784	.2857
DL298					15	.2424	.1081	.4444	.3889	.2963
DL091						18	.3500	.2564	.4103	.2667
DL081							22	.2791	.4186	.1177
DL267								21	.3333	.3030
DL092									21	.1212
DL089										12

N = 20
Mean D = 0.343 (s.e.m. = 0.009)
Mean number of bands scored = 19.1 (s.d. = 3.7)

Table A4. D scores of St. Lawrence beluga DNAs probed with 33.15

	DL339	DL185	DL187	DL188	DL189	DL342	DL192	DL239	DL334	DL341
DL339	13	.6250	.5600	.5926	.5926	.2727	.6667	.5185	.5385	.6400
DL185		19	.5807	.5455	.3636	.5000	.6061	.4849	.4375	.4516
DL187			12	.6923	.4615	.6667	.4615	.3077	.6400	.5833
DL188				14	.6429	.6087	.6429	.5714	.8148	.6154
DL189					14	.3478	.5714	.6429	.6667	.6154
DL342						9	.4348	.3478	.5455	.4762
DL192							14	.7143	.5926	.5385
DL239								14	.6667	.5385
DL334									13	.5600
DL341										12

	DL370	DL372	DL373	DL190	DL335	DL338	DL371
DL339	.5517	.6452	.5000	.6667	.6667	.3846	.5333
DL185	.5714	.6487	.4706	.6111	.4242	.4375	.5714
DL187	.6429	.4667	.5926	.5517	.3846	.2400	.5714
DL188	.6000	.6250	.4138	.5807	.6429	.4444	.8000
DL189	.4667	.5000	.4828	.6452	.7143	.4444	.5333
DL342	.4000	.5185	.4167	.4615	.2609	.3636	.6400
DL192	.5333	.6250	.5517	.5807	.5714	.6667	.6000
DL239	.3333	.5625	.3448	.5161	.5000	.5185	.5333
DL334	.5517	.5161	.4286	.6000	.5926	.3077	.6897
DL341	.4286	.5333	.4444	.5517	.4615	.4000	.5000
DL370	16	.4118	.5807	.6061	.5333	.3448	.6875
DL372		18	.4849	.6857	.5625	.6452	.5882
DL373			15	.6250	.4138	.4286	.4516
DL190				17	.6452	.4000	.6667
DL335					14	.4444	.5333
DL338						13	.4000
DL371							16

N = 17
Mean D = 0.534 (s.e.m. = 0.012)
Mean number of bands scored = 14.3 (s.d. = 2.4)

Table A5. D scores of Mackenzie Delta beluga DNAs probed with 33.6.

	DL260	DL268	DL243	DL241	DL281	DL088	DL240	DL248	DL251	DL255	DL257
DL260	19	.3226	.3902	.4651	.3889	.3636	.3500	.4706	.4118	.4242	.3158
DL268		12	.4706	.3333	.3448	.2162	.3030	.4444	.4444	.1539	.3226
DL243			22	.6522	.6154	.5532	.5581	.4324	.3784	.5050	.2927
DL241				24	.6829	.6122	.6222	.4615	.3590	.5790	.3721
DL281					17	.6191	.5333	.3750	.3750	.6452	.4444
DL088						25	.5217	.4000	.4000	.5128	.5000
DL240							21	.3888	.2778	.5143	.3500
DL248								15	.5333	.2759	.3529
DL251									15	.2758	.4706
DL255										14	.3030
DL257											19

	DL263	DL271	DL272	DL285	DL298	DL091	DL081	DL267	DL092	DL089
DL260	.2424	.5000	.5000	.3871	.4706	.4103	.4286	.3158	.3333	.3750
DL268	.2308	.2759	.3200	.2500	.3704	.3125	.4571	.2581	.3421	.4000
DL243	.2222	.4103	.4000	.4706	.5405	.6191	.5333	.3902	.5333	.2857
DL241	.2631	.4390	.4865	.4444	.5128	.6818	.6809	.3256	.6383	.3784
DL281	.2581	.4118	.6000	.4138	.5625	.7568	.7000	.3889	.6000	.5333
DL088	.3590	.3810	.4211	.3784	.4000	.5778	.6667	.4546	.4583	.4737
DL240	.1714	.4737	.5882	.5415	.5000	.6829	.5000	.4000	.5909	.4706
DL248	.2759	.4737	.5714	.4444	.4000	.4571	.4737	.4118	.3158	.4286
DL251	.4138	.5625	.4286	.2222	.3333	.3429	.5790	.5294	.2105	.4286
DL255	.1429	.3871	.5185	.4615	.4828	.5294	.5405	.3030	.5405	.2222
DL257	.3030	.3871	.3125	.2581	.2353	.4103	.4762	.3684	.4286	.3125
DL263	14	.3226	.2222	.0769	.2069	.2353	.3784	.4849	.2703	.1482
DL271		17	.5333	.4828	.1875	.4865	.4500	.5000	.4000	.4000
DL272			13	.5600	.4444	.6061	.5556	.4375	.5000	.6154
DL285				12	.4286	.5000	.3429	.1936	.5143	.4000
DL298					15	.5143	.5790	.2941	.3684	.3571
DL091						20	.6047	.3590	.6512	.4242
DL081							23	.5238	.4783	.5556
DL267								19	.3889	.3125
DL092									23	.3889
DL089										13

N = 21
Mean D = 0.424 (s.e.m. = 0.015)
Mean number of bands scored = 17.7 (s.d. = 4.2)

Table A6. D scores of St. Lawrence beluga DNAs probed with 33.6.

	DL339	DL185	DL187	DL188	DL189	DL342	DL192	DL239	DL334	DL341
DL339	21	.7308	.2927	.7143	.7660	.6471	.8261	.6471	.5500	.8000
DL185		31	.3922	.6539	.8070	.5000	.8571	.5455	.5600	.7600
DL187			20	.2927	.3913	.1818	.3556	.1212	.1539	.3078
DL188				21	.6808	.5294	.7826	.5294	.4500	.7000
DL189					26	.6154	.8628	.5128	.5333	.7111
DL342						13	.5790	.7692	.6250	.6250
DL192							25	.6316	.5455	.8182
DL239								13	.6250	.7500
DL334									19	.5263
DL341										19

	DL370	DL372	DL373	DL190	DL335	DL338	DL371
DL339	.7826	.7200	.3000	.8085	.3333	.8936	.6809
DL185	.7857	.7333	.5600	.7018	.5769	.7368	.8772
DL187	.3556	.4490	.4103	.3044	.2439	.3478	.4348
DL188	.7391	.6400	.2500	.6806	.3333	.6806	.5106
DL189	.8628	.7636	.4444	.7692	.4255	.8077	.7308
DL342	.5790	.4762	.3125	.5641	.4118	.5641	.5128
DL192	.8800	.8148	.3636	.7843	.3913	.8235	.7451
DL239	.5263	.4762	.3125	.5641	.4118	.5641	.5128
DL334	.5901	.4583	.4211	.5778	.5500	.5778	.5333
DL341	.7273	.7083	.3158	.6667	.4000	.7111	.6667
DL370	25	.7778	.4546	.7843	.3044	.8628	.6667
DL372		29	.3750	.6909	.3600	.7636	.6909
DL373			19	.3556	.6000	.4444	.6539
DL190				26	.3830	.8846	.6383
DL335					21	.3404	.6923
DL338						26	.6800
DL371							26

N = 17
Mean BSC = 0.573 (s.e.m. = 0.027)
Mean number of bands scored = 22.4 (s.d. = 4.8)

APPENDIX B.

Table B1. Summary of Mackenzie Delta beluga samples obtained from native whaling camps.

Sample	YEAR	LOCATION	SUB-POPULATION	ORIGINAL ID	SEX
DLE 081	1988	Shingle Pt.	West	9	F
DLE 088	1988	Kendall Isl.	West	15	M
DLE 089	1988	Kendall Isl.	West	16	F
DLE 091	1988	Kendall Isl.	West	19	M
DLE 092	1988	Kendall Isl.	West	21	M
DLE 241	1989	East White Fish	East	2	M
DLE 241	1989	East White Fish	East	4	M
DLE 243	1989	East White Fish	East	6	M
DLE 248	1989	Hendrickson Isl.	East	1	M
DLE 251	1989	Hendrickson Isl.	East	11	M
DLE 255	1989	Kendall Isl.	West	3	M
DLE 257	1989	Kendall Isl.	West	5	M
DLE 260	1989	Shingle Pt.	West	4	M
DLE 263	1989	Shingle Pt.	West	5	U
DLE 267	1989	Tuktoyuktuk	East	7	F
DLE 268	1989	Tuktoyuktuk	East	9	F
DLE 271	1989	West White Fish	West	1	F
DLE 272	1989	West White Fish	West	3	F
DLE 281	1990	East White Fish	East	3	M
DLE 285	1990	Tuktoyuktuk	East	2	U
DLE 298	1990	Shingle Pt.	West	10	M

(To be submitted)

SKIN BIOPSY SAMPLING OF BELUGA CARCASSES: ASSESSMENT OF BIOPSY DARTING FACTORS FOR MINIMAL WOUNDING AND EFFECTIVE RETRIEVAL ON BELUGA WHALES

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ABSTRACT

Different combinations of biopsy tip and stop collar sizes were tested on fresh beluga carcasses to determine factors affecting the success of retrieval and the extent of wounding. Tips with smaller diameters and longer length were more likely to retrieve a skin sample ($p < 0.05$ and $p < 0.10$ respectively) while the force of impact, a function of draw weight of the crossbow and distance from the target, had a significant effect on the severity of wounding ($p < 0.05$). The samples obtained from all biopsy darts tested yielded sufficient amounts of DNA for genetic analysis. The highest DNA yield was found in the germinativum spinosum layer of the skin.

Key words: biopsy darting, wounding, beluga, DNA yield

Recent developments in molecular biology have enabled us to gain invaluable information from a small amount of tissue. Skin tissue can be successfully obtained from whales with the technique of biopsy darting. The DNA collected can be used to establish tissue cultures (Lambertsen 1987; Matthews et al 1988), to determine gender of individuals (Winn et al. 1973), to determine genetic structure of populations (Baker et al. 1990) or to determine contaminant levels (Brown et al. 1991). Biopsy darting methods utilize a projection unit (usually a crossbow) and a biopsy dart mounted on a projectile (usually an arrow). The dart includes a hollow stainless steel shaft with an aluminium collar (Lambertsen 1987; Matthews et al. 1988) at its base to prevent deeper penetration of the skin. The arrows are freed upon contact by the resilient compression of the underlying blubber. The sample is retained in the tip by a device such as a hook or barbs. A tissue sample is thus cut on penetration and torn on rebound.

Several biopsy systems successfully obtain small skin samples from humpback whales, fin whales, sperm whales, minke whales and others (Hoezel et al. 1983; Matthews et al. 1988; Lambertsen 1987, Whitehead et al. 1990; Arnason et al. 1985). Whale biopsy darting research has been limited largely to the design of equipment for effective retrieval of tissue and specific behavioural responses to darting for each species under study (Palsboll et al. 1991; Brown et al. 1991).

Though some researchers have attempted biopsy darting of beluga whales (Brennin 1992), there are no published data on the factors contributing to the successful biopsy darting of this species. Furthermore, no cetacean study, to our knowledge, has documented the wounding created by darting or focused on the factors influencing the extent of wounding.

The necessity to develop a darting program on beluga whale populations is becoming apparent. Beluga tissues required for genetic studies have typically come from native harvests or beached animals. This first type of sampling may be biased as hunted whales travelling in groups may represent family units. Obtaining samples from beached animals may also be biased if closely related individuals are more susceptible to lethal pathogens. Biopsy darting offers a possible alternative to these sampling methods and would also allow skin tissue collection from beluga populations where samples are not otherwise available, as for Arctic populations that are not hunted.

The objective of this study was to develop a reliable method for collecting skin samples from biopsies of free-ranging beluga whales and document the factors that may affect the extent of wounds left by biopsy darting. Initial attempts were made to evaluate the impact of darting on St. Lawrence beluga carcasses. These attempts gave inconclusive results because the skin was highly degraded by the time the darting could be performed on the beached whales. To better simulate the impact of darting of live free-ranging whales we darted freshly killed beluga whales from the Mackenzie Delta estuary.

METHODS

Field work

Research was carried out at Shingle Point, Yukon Territory, Canada during July 1992. Seven whales were darted repeatedly within a few hours of death after being landed by native hunters. An eighth whale was darted within 48 hrs of death, having been struck and lost by hunters but beached two days later. Samples, in most cases, were taken just below the dorsal ridge. In one case the whale was beached on its backside and three darting attempts were made on its abdominal area, about 60 cm below the dorsal ridge. The samples were preserved in supersaturated salt solution at ambient temperature (0^0-20^0 C) until processing.

A darting attempt was considered successful when the dart rebounded off the whale and a significant plug of skin was still attached to the hook when the cylindrical shaft was removed. Wounds were qualitatively described on a scale of 0 to 4. A wound was recorded as minimal (0) when only the puncture wound was created by the cylindrical cutting tip, leaving a clean entry wound (Fig 1). Intermediate wounds (1,2,3) were described by the amount and depth of tearing surrounding the puncture wound. A wound was described as maximal (4) when very heavy tearing of the epidermis and dermis accompanied the puncture wound, leaving a gashing wound.

Because it was impossible to predict the number of whales that would be available for darting, the design of this study was not to test every possible combination of factors but to try to focus in on combinations that would effectively retrieve a sample

while minimizing wounding. It ensues that the sample size for the different classes may vary greatly.

Two types of crossbow were used: a Barnett Wildcat with 23, 45 or 68 kg draw weights and an Excalibur Wolverine compound bow with 45 kg draw weight. The 45 kg draw weight of the two models of crossbows is not a comparable measure of their firing power. The Excalibur compound bow is more powerful than the standard crossbow. Its firing power is roughly equivalent to the 68 kg draw weight of the Barnett Wildcat (B. Throubridge, pers. comm.). For statistical treatment, the Excalibur 45 kg draw weight was estimated at 68 kg. The firing range was constrained by the topography of the landing area. The distance between the archer and the whale being darted varied from 1.5 to 15 meters.

The cylindrical stainless steel tips tested were similar in design to those described in Brown et al.(1991)(Fig. 2). Six different tip length and diameter combinations were tested in combination with six stops of different diameters (Table 1). The aluminium stops were of one design (Brown et al. 1991).

The retaining device for the tissue was either a straightened #6 cod hook (Brown et al. 1991), or a modified hook with a series of barbs on it. These were glued to an insert which was threaded to fit the dart on one end and the arrow on the other.

The angle at which the dart penetrated the skin was estimated by placing the dart back into the wound after each attempt and measuring the angle with a protractor. An angle of 90^0 meant that the dart penetrated the skin at a perpendicular plane to the whale's skin surface.

To evaluate if the darts retrieved sufficient tissue for genetic analysis, DNA from 25 skin plugs obtained from the different darts tested, was extracted and tested for quality using techniques described in Patenaude et al. (in prep.). DNA was digested with EcoRI endonuclease enzyme and fragments separated by size on a 1% agarose gel by electrophoresis. DNA concentrations were estimated with a fluorometer.

We also evaluated the quality of DNA found in the different dermal layers of beluga skin. A small amount of skin was cut with a razor in three sections. The first layer included the stratum spinosum and the stratum corneum of the epidermis (Fig. 3). The second layer included the stratum germinativum of the epidermis and the papillary layer of the dermis while the third layer was composed of the reticular layer of the dermis. The DNA extracted from each layer was digested with EcoRI and run on 1% agarose gel to examine DNA quality.

Statistical analysis

We used Pearson's Chi-square test (SAS JMP) to test for correlation between independent factors and wound size/success of retrieval. The draw weight of projection unit cannot be treated as an actual measure of force impact because it depends on the distance of string travel as well as the distance travelled by the arrow. The distance of string travel being held constant, we used the distance travelled by the arrow as a weighted variable in a function (force of impact) which takes into account draw weight and distance. An arrow will retain about 90% of its kinetic energy at close range (10 meters) (B. Troubridge, pers. comm.). We estimated a one percent loss of power per

meter. The function of force of impact (Fi) was calculated as

$$Fi = DW - 0.01*D*DW$$

where DW is the draw weight of the crossbow and D is the distance travelled by the arrow. This function was modelled with a logistic regression (Table 3). We used a Chi-square test to evaluate how well the model fit the data.

We used a two-way ANOVA to test if there was a significant difference in DNA yield from the different dart diameters and lengths.

RESULTS

A total of 40 biopsies were made on 8 whales. On 6 attempts the arrow did not rebound but stayed in the whale and on two occasions tip #7 broke on impact. No specific factor was associated with either result. There was perceptible flight change when a tip was combined to a stop of 38 mm in diameter at 12 m distance with a 45 kg draw weight. Tips with smaller diameters were significantly more likely to retrieve a sample ($X^2_{[2]}=9.7$, $p<0.05$). The percent of retrieval ranged from 95% (18/19) for a 5 mm diameter tip, through 86% (12/14) for 6mm diameter tip to 43% (3/7) for a 7mm diameter tip (Table 3).

The probability of retrieval did not vary significantly according to tip length ($X^2_{[1]}=2.8$, $p=0.09$). Nevertheless, the longer tip (25 mm) never failed to retrieve a sample (10 attempts) while the 20 mm tip successfully retrieved a sample only 77% (23/30) of the time suggesting a trend (Table 4). The other factors tested did not significantly affect the probability of retrieval (Table 5).

The force of impact, a function of the draw weight of the crossbow and the distance travelled by the arrow, had a significant effect on the type of wound induced ($X^2_{[36]}=20.6$) (Fig. 4). As expected, the wound was significantly more severe as the force of impact increased. Other fcators were not significantly correlated to wound type (Table 5).

The skin plugs obtained from all biopsy dart sizes tested yielded sufficient amounts

of DNA for DNA fingerprinting and restriction fragment length polymorphism (RFLP) analysis. DNA yield was found to vary according to tip diameter ($F = 26.0$, $p < 0.01$). The maximal yield, as expected, was from the tip of largest diameter (Table 6). The Tip length did not influence the DNA yield ($F = 3.6$, $p = 0.07$).

A difference in DNA yield and quality was observed for different skin layers. The highest DNA yield for an equal weight (0.50 g) of tissue was observed in the layer including the germinativum spinosum of the epidermis and the papillary layer of the dermis (Fig. 5). These results are in accordance with Palmer and Weddell (1964) who reported a high mitotic rate in the germinativum layer in cetaceans. DNA was slightly degraded in the outermost section containing the epidermis and contained a glycogen precipitate formed during extraction (Fig. 5). Endonuclease cleavage was possible for all three sections.

The statistical analysis was based on the entire data set, including the three darting attempts in the lower mid-dorsal section though it is unlikely this area would be darted on free-ranging belugas. Excluding these data points reduces the sample size and changes the significance of two results . The probability of the tip diameter and tip length affecting retrieval become $p = 0.08$ ($X^2_{[2]} = 5.2$), and $p = 0.13$ ($X^2_{[1]} = 2.3$) respectively. The discussion is based on results obtained with the entire data set.

DISCUSSION

Biopsy darting is an important tool for obtaining samples used in molecular analyses, sexing of individuals and in contaminant analyses. Such analyses are valuable to the management and conservation of belugas. We have investigated factors that may influence the likelihood of success in retrieval and the severity of wounding during biopsy darting.

Based on our results, we recommend the following for darting of free-ranging beluga whales: We suggest using a tip of 5mm in diameter and 25 mm in length. This tip gave an average amount of DNA (66 ug) sufficient for DNA fingerprinting, RFLP and mitochondrial DNA analysis. Its smaller diameter and longer length results in an increased probability of retrieval. This may be due to the intrinsic nature of beluga skin. A smaller but longer dart may reach deep enough into loose connective tissue (Bonin and Vladykov, 1949) and facilitate tearing of a small surface area. Although one of these tips broke, increasing the wall thickness of the dart or fabricating the dart with a stronger material such as hardened tool steel may eliminate the problem of breaking on impact.

We suggest the straightened #6 cod hook as a retaining device. Both hook types had the same probability of retrieval but the #6 straightened cod hook is more easily and inexpensively manufactured. The diameter of the stop should be kept to a minimum size. As reported by others (Pallsboll et al. 1991), an increased stop size

also increases the wind resistance and may alter the flight pattern of the arrow.

Most of the variation observed in wound type was correlated to the force of impact. As expected, a more powerful blow increases the severity of the wound. We suggest using a less powerful bow such as the Barnett Wildcat with a draw weight of 23 kg to minimize wound size. Belugas are rapid swimmers and expose a small target when surfacing leaving little time to the archer. Darting should be done opportunistically at close distance to avoid the need to adjust aim for flight curve.

Resistance to the use of biopsy darting lay in perceptions that it may be excessively invasive to the whales. The IWC (1991) suggest that there are no indications of short-term or long-term detrimental effects caused by biopsy darting. Behavioural data on right whales and on humpback whales suggest that darting elicits a momentarily response to pain or surprise, the extent of which depends on the species under study (Brown et al. 1991; Weinrich et al. 1991). There is no doubt that biopsy darting entails physical costs to the whale. Therefore, efforts should be made to minimize these costs by limiting the extent of wounding and maximizing the benefits by increasing the rate of success of obtaining a sample at every darting attempt and samples should yield sufficient amounts of DNA for multiple genetic analyses.

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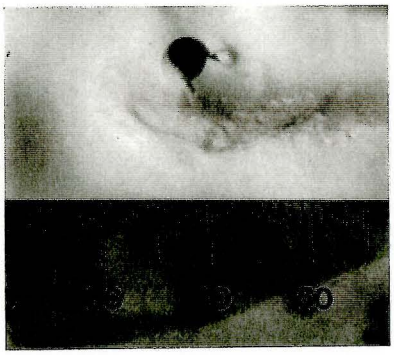
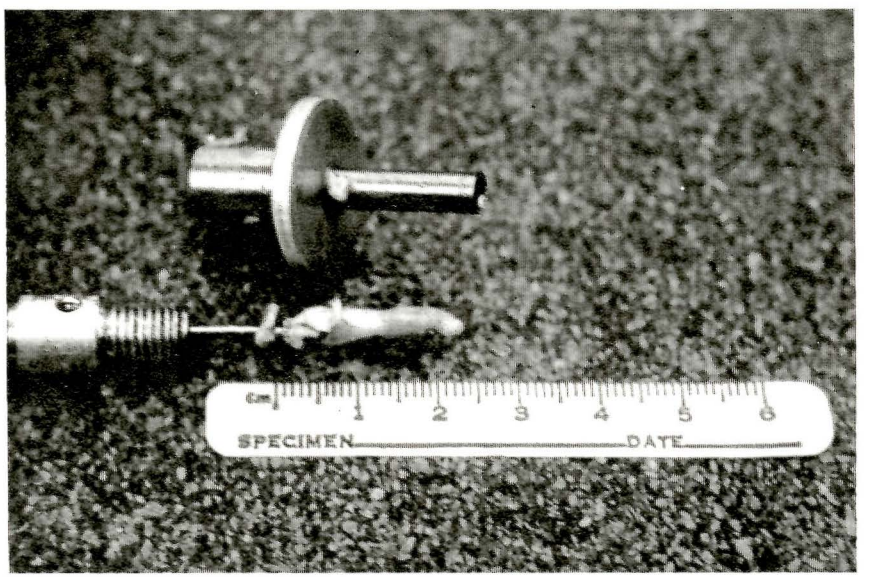
Figure 1. Wound types after biopsy darting of a beluga carcass. Wound type 0 (left) is characterized by a clean puncture wound while wound type 4 (right) shows heavy tearing of the epidermis and dermis.



1cm

Figure 2. Biopsy dart #5 with aluminium stop. The hook retaining the skin plug is glued to an insert threaded to fit the dart.


1 2 3 4 5 6
SPECIMEN
DATE

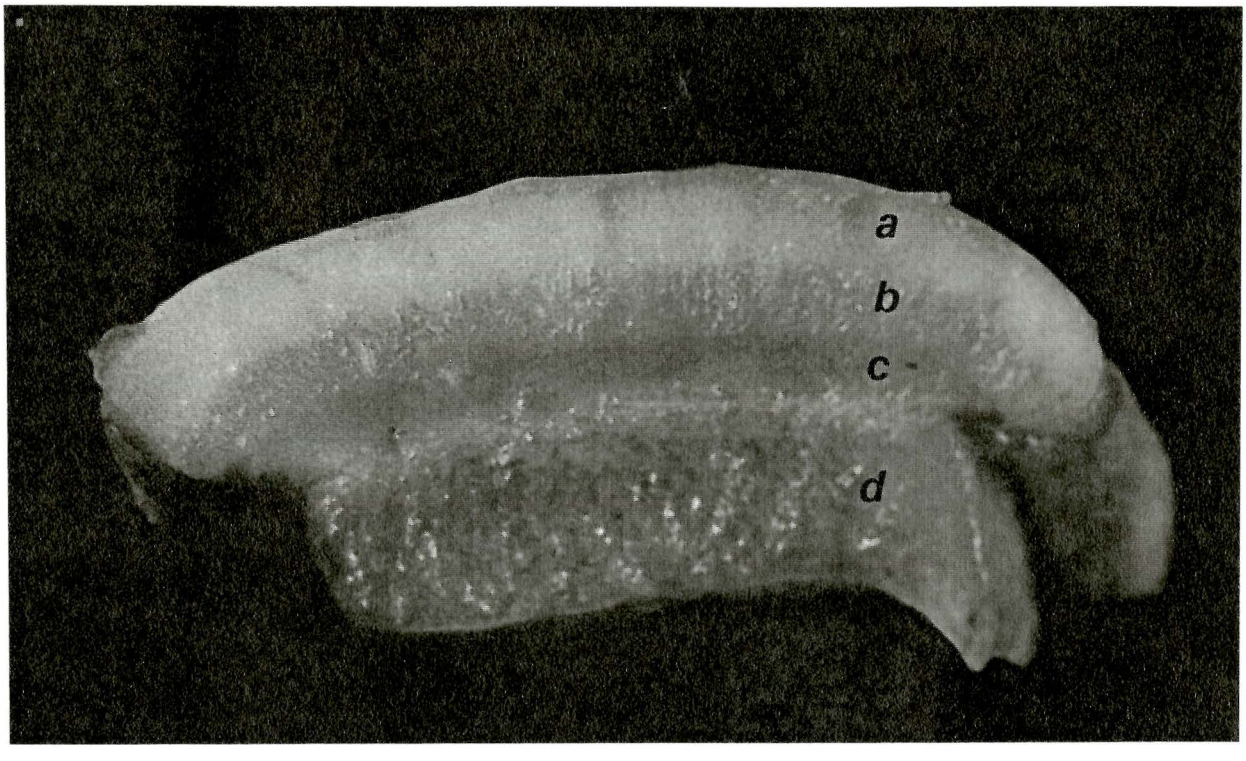

a
b
c
d
1cm

Figure 3. Cross section of beluga skin with a) area of the epidermis and the stratum corneum of the dermis, b) area of the stratum germinativum of the epidermis and the papillary layer of the dermis, c) the reticular layer of the dermis and d) part of the hypodermis.

Figure 4. Logistic regression of the force of impact on the type of wound. A set of probability curves partition the probability axis (left) at each point on the x axis (force of impact). At any point on the y axis (type of wound), the probability associated with each type of wound is the vertical distance on the probability axis corresponding to that response.

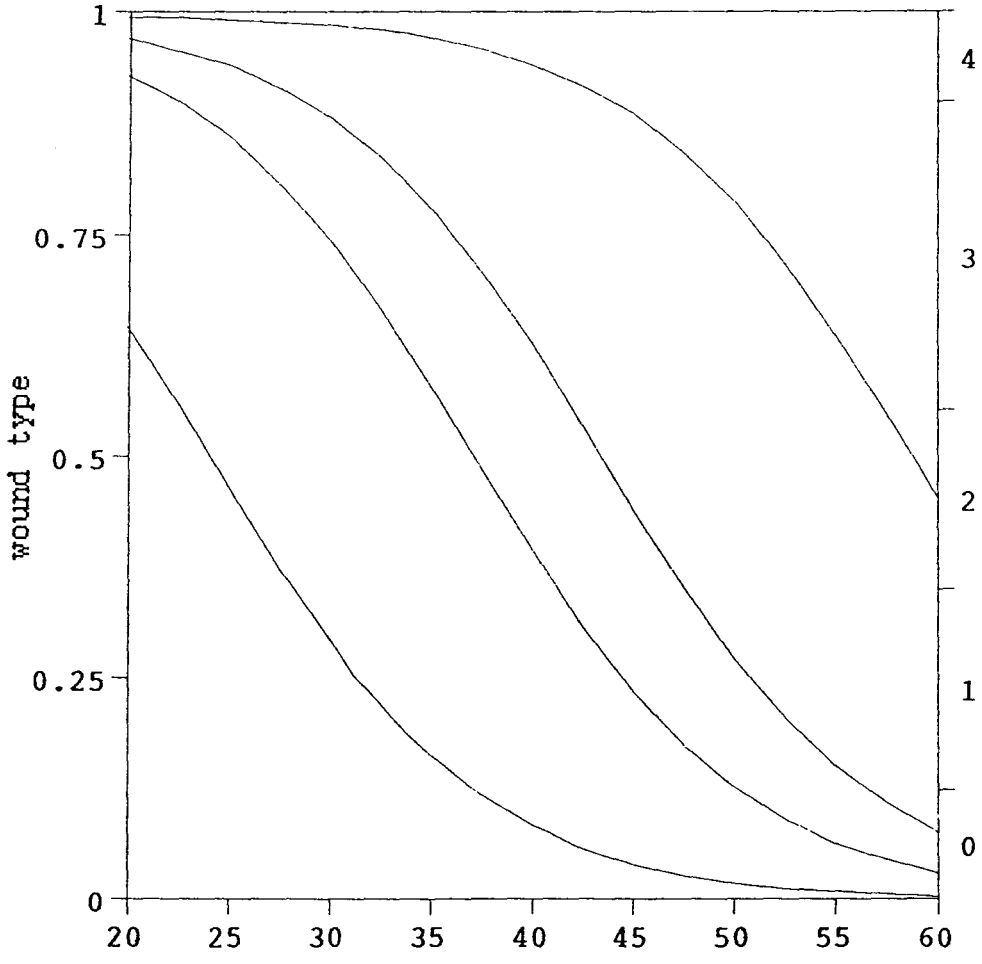

1
0.75
0.5
0.25
0
wound type
4
3
2
1
0
20
25
30
35
40
45
50
55
60
Force of impact

Figure 5. Equal amounts of DNA (0.50 *u*g) from three beluga skin layers. A) DNA extracted from the stratum corneum and stratum spinosum layers of the epidermis shows a white glycogen precipitate, B) the layer composed of the stratum germinativum of the epidermis and the papillary layer of the dermis shows a large amount of precipitated DNA, C) the reticular layer of the dermis shows a much smaller amount of precipitated DNA.

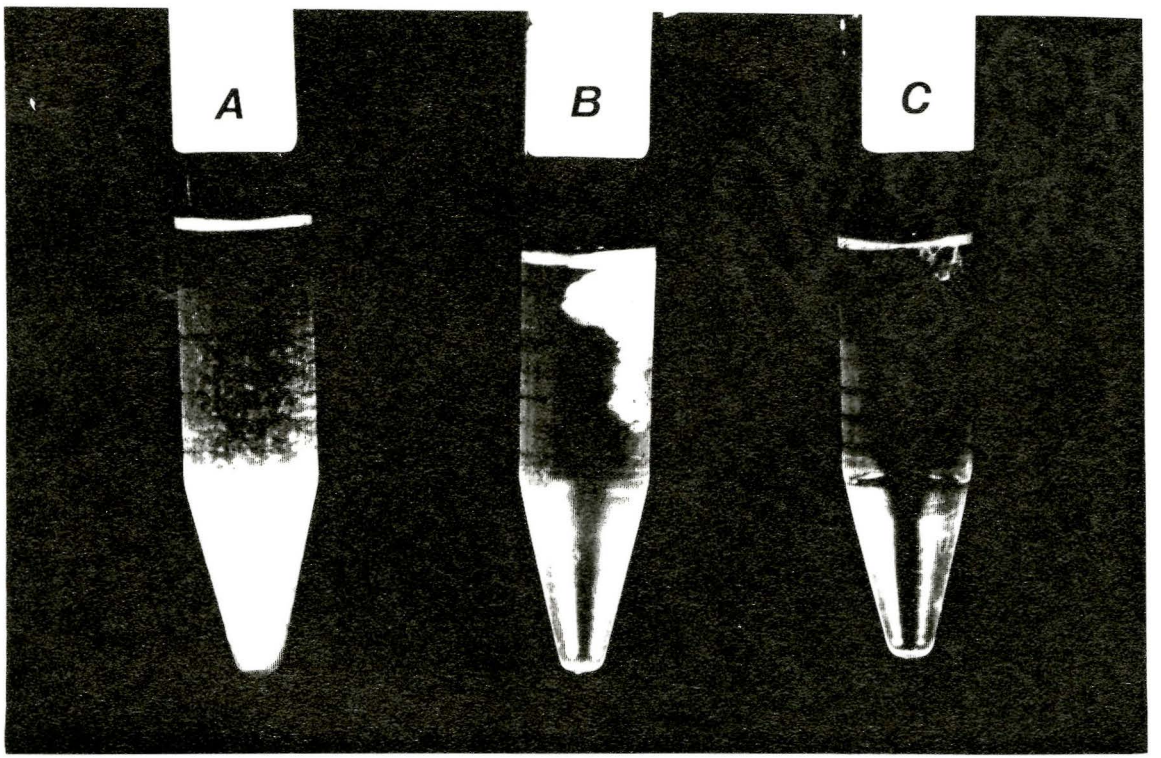

A
B
C

Table 1. Length and diameter of the different tips and stops tested on beluga carcasses.

Tip #	diameter (mm)	length (mm)
2	7	20
3	6	25
5	6	20
6	5	25
7	5	20

Stop #	
1	38
2	35
3	32
4	26
5	22

Table 2. The force of impact (Fi) function of the distance travelled by the arrow and the draw weight of the crossbow.

	Distanc (m)					
Draw W. (kg)	1.5	3	6	9	12	15
23	22.7	22.3	21.6	20.9	20.2	19.6
45	44.3	43.7	42.3	41.0	39.6	38.3
68	66.7	66.0	63.9	61.9	59.8	57.8

Fi= DW - 0.01*D*DW, DW = draw weight of the crossbow and D = distance travelled by the arrow.

Table 3. Response counts of success of retrieval for different tip diameters

	Tip diameter (mm)			Total
Retrieval	5	6	7	
No	1	2	4	7
Yes	18	12	3	33
Total	19	14	7	

Table 4. Response counts of success of retrieval for different tip lengths

	Tip length (mm)		Total
Retrieval	20	25	
No	7	0	7
Yes	23	10	33
Total	30	10	40

Table 5. Summary of the effect of the factors tested on the success of retrieval and the type of wound inflicted.

	Draw weight	Angle of penetration	Tip diameter	Tip length	Stop diameter	Hook type
retrieval	n.s.	n.s.	**	*	n.s.	n.s.
wound	**	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. non significant, ** $p<0.05$, * $p\leq 0.1$

Table 6. DNA yield extracted from beluga skin plugs obtained with different tip sizes.

tip #	tip (mm)		ave. yield	min. yield	max. yield	s.d.	n
	diam.	length	*u*g	*u*g	*u*g		
7	5	20	33.4	20	65	12.2	11
6	5	25	66.0	48	84	18.0	2
5	6	20	58.6	40	80	15.8	5
3	6	25	76.0	55	111	21.9	4
2	7	20	94.7	80	109	11.8	3

CONCLUSION

The role of population genetics in the management and conservation of threatened or endangered wildlife populations is becoming increasingly important. As loss and degradation of habitat affects population numbers, the issue at hand can no longer be limited to ecological and geographical concerns. Most of the research on St. Lawrence belugas has focused on extrinsic factors such as habitat degradation and contamination by toxic chemicals as the prime hypothesis for their lack of recovery. This research has shown that an intrinsic factor such as the genetic make-up of the population may be contributing to its lack of recovery. With this added information in hand, efforts geared towards the conservation of the St Lawrence beluga may need to be redefined. The ultimate goal of conservation is to establish a large and genetically healthy population in a restored and protected natural habitat. This latter goal is somewhat met by the creation of a marine park that encompasses prime beluga habitat. In view of the risks of inbreeding depression suggested in this study, future management decisions should be geared towards increasing the genetic variability of the St. Lawrence beluga population.

The technique of biopsy darting offers an attractive method of sampling beluga whales. By using small diameter darts and low crossbow poundage it becomes possible to minimizing the physical impact of darting while successfully obtaining sufficient amounts of DNA for genetic analysis invaluable to the management and

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