Conservation Genetics of the St. Lawrence Beluga Whale (*Delphinapterus leucas*)

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Abstract

Beluga whales (*Delphinapterus leucas*) migrated to the St. Lawrence Estuary near the end of the Holocene glacial retreat, when dry land emerged. The population currently inhabiting the St. Lawrence shows strong site fidelity, which is generally common with belugas. Decades after a ban on the hunting of the beluga whale in 1979, the lack of recovery noticed in the St. Lawrence Estuary (SLE) population has puzzled researchers who study beluga whales. The St. Lawrence Estuary has been known for its industrialization, as well as its large amounts of vessel traffic, contributing to entanglements, as well as pollution to the river and its inhabitants. The current population of belugas inhabiting the SLE are ~1000 or less, and are declining, which is concerning because the species as a whole are endangered. This research project focuses on assessing the threat of inbreeding and low genetic diversity on the small, isolated population of the SLE beluga. To estimate the degree of inbreeding, as well as the loss of genetic diversity within the SLE beluga, Arctic samples from Nunavik were genotyped using multiplex PCR and capillary electrophoresis for the creation of microsatellite profiles for each individual. The microsatellite profiles of the Nunavik individuals were then compared to with those from the SLE beluga that had previously been genotyped. The effective population size (Ne) was also estimated for the SLE population.

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Introduction

Genetics plays a crucial role in species conservation (Kardos. 2021). Conservation genetics is a field that incorporates the use of genetic principles and methods in order to advance the preservation of biodiversity (Kardos. 2021). Some of the major genetic issues that are the focus of conservation genetics include the deleterious effects of inbreeding on reproduction and survival, as well as the loss of genetic diversity and its impact on the ability of populations to adapt in response to environmental changes (Frankham et al., 2010). Conservation genetics aims to minimize the risk of extinction from genetic factors (Frankham et al., 2010).

Conservation Genetics and Small Populations

Human-related factors, such as over-hunting, pollution, and industrialization can reduce abundance to sizes that are susceptible to stochastic events, or naturally occurring fluctuations that are experienced by small populations (Frankham et al., 2010). Stochastic effects are chance events that occur commonly in nature; they can be unpredictable events that effect population and community dynamics (Lesage et al., 2014). Effects from stochastic events could include environmental, demographic, and catastrophic factors that increase the risk of extinction in small populations (Frankham et al., 2010). This means that even if the original cause of the population decline ceases, there may still be problems that can result simply by having a small population size.

Conservation genetics is a diverse field that uses genetic principles to characterize, and advance the preservation of biodiversity, especially in populations that are at risk (Amos & Balmford. 2001). There are many threats that a small population can face affecting their long-term viability, such as potential loss of genetic diversity over time, inbreeding depression, as

well as the increased susceptibility to environmental and demographic stochasticity (Uzans et al., 2015). Environmental stochasticity refers to random variations in environmental conditions over time, and large deviations from typical environmental conditions at any time could have detrimental effects on a small population (Wilson & Primack. 2001). Demographic stochasticity refers to the random fluctuations in population size due to the birth and death of individuals in a population being discrete and probabilistic events (Wilson & Primack. 2001). As a result, a small population's risk of extinction increases as a result of the stochastic variation it experiences (Uzans et al., 2015, Caughley. 1994). Genetic factors can exaggerate this "demographic stochasticity" as well, and add to additional threats, with one example being the loss of genetic variation that leads to mates who become increasingly closely related (Kardos. 2021).

The objective of conservation biology in particular is to preserve species as dynamic entities that are capable of evolving in order to adapt with environmental change, and it is important to research small populations that are not growing, especially if they are listed as a species at risk (Caughley. 1994). Understanding the natural forces that drive evolutionary change is paramount in protecting and managing endangered populations before they are pushed toward extinction (Frankham et al., 2010).

Inbreeding

Isolation and small population size will eventually lead to inbreeding (the mating between relatives), which in-turn results in the expression of recessive deleterious alleles that would otherwise be masked in the heterozygous state (Frankham et al., 2010). At the same time, as population size decreases the magnitude of genetic drift (which is the random change in allele frequencies over time) increases, resulting in an increasing rate at which genetic diversity is lost (Frankham et al., 2010). This loss of genetic variation can increase extinction risk by limiting the

ability of a population to adapt to future environmental changes (Kardos. 2021). For example, in the research paper by Acevedo-Whitehouse et al., 2003, California Sea Lions were scored for 11 highly polymorphic microsatellite DNA markers (Acevedo-Whitehouse et al., 2003). The results showed that sick animals had a higher than normal parental relatedness (Acevedo-Whitehouse et al., 2003). For example, individuals with carcinoma showed a high internal relatedness (Acevedo-Whitehouse et al., 2003). More inbred individuals also take longer to recover from disease, infections, etc., than do non-inbred individuals (Acevedo-Whitehouse et al., 2003).

Another instance where inbreeding becomes a concern in conservation genetics involves a population of greater prairie chickens. The Illinois population of greater prairie chickens is evidence showing that inbreeding adversely effects wild populations. For example, in 1992 a greater prairie chicken population in Illinois had declined to around 200 individuals and had failed to recover following the restoration of their habitat (Frankham et al., 2010). The greater prairie chicken population showed clear evidence of inbreeding depression in terms of reduced fertility and hatchability of their eggs (Frankham et al., 2010). In small populations, the frequency of homozygotes is higher, and due to the effects of deleterious alleles becoming homozygous, inbred offspring almost always show reduced reproduction and survival, as compared to non-inbred counterparts (Kardos. 2021). The overall effects of inbreeding can cause the population growth rate to decline over time as offspring in the small population become increasingly inbred (Kardos. 2021).

The proportion of individuals that survive and reproduce can fluctuate through time just by random sampling effects where there is only a small number of effective breeding individuals (Kardos. 2021). As a result, small populations are more likely to become extinct due to their high variability in viability of reproductive offspring the smaller that the population gets. When

populations are small, inbreeding and genetic drift both have the tendency to increase as the number of individuals that are contributing offspring to the next generation begin to decrease (Pekkala et al., 2014).

Genetic Drift

Genetic drift refers to the random fluctuations in the frequency of alleles over time based on chance events, either causing traits to become dominating or removed from a population (Pekkala et al., 2014). At the same time, the average fitness of a population will be expected to decrease from generation to generation as the level of inbreeding, or homozygosity, increases (Pekkala et al., 2014). In the wild as well as experimental studies, a positive relationship between population fitness and heterozygosity is often observed (Pekkala et al., 2014). For example, the Florida panther suffers from low genetic diversity and inbreeding-related defects, such as poor sperm quality and quantity, as well as morphological abnormalities (Frankham et al., 2010). Inbreeding and loss of genetic diversity are two fundamentally different consequences of having a small population size as far as genetic variation is concerned (Frankham et al., 2010).

Low Genetic Diversity

As predicted by the fundamental theorem of natural selection in evolutionary biology, populations with low genetic variation will have reduced evolutionary potential (Frankham et al., 2010). Therefore, populations with decreased genetic diversity will be less likely to be able to adapt to future environmental changes (Frankham et al., 2010). Genetic variation allows populations to tolerate a wide range of environmental extremes, and so the loss of genetic diversity reduces the ability of a population to evolve in order to cope with environmental

change (Frankham et al., 2010). The loss of genetic variation means that there is less gene migration (the transfer of genetic material from one population to another) for selection to act upon, thus reducing the ability of the population to adapt to environmental changes such as global warming, drought, and invasive species, for example (Kardos. 2021).

Individuals in small populations have shown to express a low genetic diversity, and or decreased major histocompatibility complex (MHC) diversity (Lair et al., 2016). The loss of MHC diversity was found to decrease antiviral immunity in the small population of free-range Tasmanian Devils *(Sarcophilus harrisii)* who expressed neoplasms that included facial tumours due to transplanted neoplastic cells, due to the cells of the individual being unable to distinguish viruses as other versus the self (Lair et al., 2016). Genetic factors and contaminants may also be involved in viral oncogenesis - viruses that cause and give rise to cancerous tumors (Lair et al., 2016). For example, in the large and increasing population of California sea lions, specific MHC alleles as well as a high amounts of PCB (polychlorinated biphenyl) contaminants contributed to their viral oncogenesis (Lair et al., 2016). One of the trademarks of the immune system is its ability to differentiate between the 'self' and 'non-self' (Ober et al., 1998). The term 'non-self' is defined as anything that is detectably different to that of an animal's own constituents (Ober et al., 1998). This phenomenon was described in the Cheetah (*Acinonyx jubatus)* population which underwent a severe population bottleneck that lead to genetically identical individuals, manifesting a lack of rejection of skin transplants through the loss of genetic diversity at the MHC locus (Garner et al., 2020).

There is direct evidence that inbreeding and the loss of genetic diversity increase the risk of extinction for wild populations (Frankham et al., 2010). The most compelling body of evidence for such a statement is that of the butterfly populations in Finland (Kankare et al.,

2004). In 1995, forty-two butterfly populations in Finland were typed for microsatellite genetic markers, with their fates recorded in the following year (Kankare et al., 2004). Of the forty-two populations, thirty-five survived until the autumn of 1996, and seven populations went extinct (Kankare et al., 2004). Extinction rates were higher for populations with lower heterozygosity, which is an indication of inbreeding, even after accounting for demographic and environmental stochasticity (Frankham et al., 2010).

Beluga Whale (*Delphinapterus leucas)*

The Beluga whale, *Delphinapterus leucas,* is a small toothed whale of the *Monodontidae* family, and is adapted to artic and sub-arctic conditions (DFO. 2012). The beluga is characterized by their absence of a dorsal fin, their thick skin, as well as their tough dorsal ridge that is used to break ice and preserve heat (DFO. 2012). They possess a rounded head, broad flippers, and have a structure known as a melon on the dorsal surface of the head that is filled with lipids which aids in echolocation (DFO. 2012).

Adult belugas have white skin, while juveniles are characterized by their greyish brown skin (DFO. 2012). These cetaceans (marine mammals of the order *Cetacea*), have a long life expectancy of about 30-60 years, and some carcasses washed ashore have been determined to be in the 80-year range (DFO. 2012). As well, belugas have a delayed sexual maturity and low reproductive rate – they only produce one offspring at a time, and in 3-year reproductive cycles (DFO. 2012).

Lack of Recovery of the St. Lawrence Estuary Beluga Whale

Despite the reduction in the hunting of Belugas since 1945, as well as the prohibition of hunting them in 1979, the St. Lawrence Estuary (SLE) population has failed to recover to its pre-

exploitation numbers, indicating that unintentional factors are limiting recovery (Lair et al. 2016). What factors are limiting recovery are unclear, but hypothesis include high loads of chemical contaminants, low genetic diversity, and habitat degradation to increased human activities. In addition to not showing signs of recovery, they have actually been in decline for about 10 years, which appears to be due to an increased rate of juvenile mortality (Lair et al., 2016).

Belugas migrated to the St. Lawrence Estuary near the end of the Holocene glacial retreat, when dry land emerged (Lesage et al., 2014). In 2004, SLE beluga population was assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as endangered, meaning that the beluga species faces a high risk of immediate extinction (COSEWIC. 2014). Listing a species or sub-species as endangered provides a scientific foundation for national and international legal protection from exploitation and trade, which will hopefully lead to actions of recovery (Frankham et al., 2010).

In spite of over 4 decades of protection from hunting, the SLE beluga population is comprised of about 900 individuals (Gosselin. 2015). The population has not grown much or showed any significant change in abundance over the last 18+ years (DFO. 2012). The population growth rate of the SLE beluga population has been estimated with a great deal of uncertainty at 1%, which is a very slow growth rate for a population that is no longer being harvested (DFO. 2O12). Hunting of belugas drastically reduced the population, causing a genetic bottleneck (DFO. 2012). A genetic bottleneck is an extreme example of genetic drift where the size and genetic diversity of a population is severely reduced by an event such as hunting; killing many individuals and leaving a small random assortment of survivors behind (DFO. 2012).

Because SLE belugas have relatively low genetic diversity, it has been proposed that the population may have a genetic susceptibility to cancers (Lair et al., 2016). For example, the population of California Sea Lions, indicates that inbreeding seems to become an important factor in the susceptibility to long-lived parasites by showing that more inbred individuals harbour a wider range of helminth infections, while also taking longer to recover than less inbred individuals (Acevedo-Whitehouse et al., 2003). The genetic diversity of the SLE beluga is very low as compared to other Canadian Beluga populations, such as those in the Arctic (DFO. 2012). This is suggestive that either genetic drift, inbreeding, or both combined, have influenced the genetic characteristics of the population in the St. Lawrence Estuary (DFO. 2012).

Estimating Inbreeding

Internal relatedness (IR) will be used to estimate inbreeding for the SLE beluga, an isolated population that did not migrate to the Arctic (COSEWIC. 2014). Internal relatedness weights heterozygosity values based on the characteristics of alleles, rather than their loci; therefore, an increase in heterozygosity should show a low internal relatedness (Amos et al., 2001). When closely related parents mate, it is very common for their offspring to show reduced fitness, and the IR metric can gain information from the frequency of alleles for which an individual is homozygous (Amos et al., 2001). IR is an alternate measure of estimating inbreeding that has moved from the use of simple heterozygosity as a genetic method for estimating levels of inbreeding using microsatellites (Bean et al., 2004). IR is based on allele sharing, where the frequency of every allele counts toward the final inbreeding value (Bean et al., 2004). In this way, the method weights shared rare alleles more heavily than shared common

alleles in order to provide a direct estimate of the relatedness between an individual's parents (Bean et al., 2004).

Effective population size

The effective population size (N_e) is an important parameter in conservation genetics that determines how many individuals in a population will contribute alleles to the next generation of offspring (Bouzat. 2010). Effective population size translates the census size of a real population into the size of an idealized population showing the same rate of loss of genetic diversity as the population under study by quantifying the magnitude of genetic drift and inbreeding in real-world populations (Wang et al., 2016).There is a relationship between having a small N_e, low genetic diversity and extinction risk (Bouzat. 2010). The result of having a small number of individuals that will contribute alleles to further generations creates a situation where there is an increased likelihood for the genetic diversity of the population to decrease (Bouzat. 2010). Decreased genetic diversity in a small population with an even smaller N_e, in turn could have decreased fitness as a result, as well as decreased evolutionary potential (Wang et al., 2016). All of these instances create a domino effect that results in an increased risk of extinction (Bouzat. 2010). Small populations and their genetic consequences depend on their N_e, rather than on the actual number of individuals in the population (Wang et al., 2016).

Microsatellites

Microsatellites have long been used in population genetics studies, as they have a high mutation rate that can inform about recent population divergence (Buchanan et al., 1996). Microsatellites are short tandem repeats of about 1-6 base pairs in length that are abundant in the genome (Ellegren. 2004). Microsatellites have proven to be successful in detecting population

structure among closely related populations (Akemi et al., 2012). Conservation geneticists often use microsatellite loci as genetic tools to estimate population statistics such as heterozygosity and allelic diversity, and to determine if a population went through a genetic bottleneck (Akemi et al., 2012). The high mutation rate of microsatellites can inform us about recent population divergence, as their use has proven successful in detecting population structure among closely related populations (Akemi et al., 2012).

SLE belugas can be differentiated from all other Canadian beluga populations using microsatellites, as they have shown to be the most genetically divergent population of Belugas in Canada based on their nuclear markers (COSEWIC. 2014). The low nuclear genetic diversity that is observed in SLE belugas is similar to that observed in other populations of mammals that are isolated (COSEWIC. 2014).

Research Goal

Research into the genetic comparison between the SLE beluga population and arctic beluga individuals will aid in answering a large-scale question that considers if low genetic diversity, and or inbreeding, are at least partially responsible for the lack of recovery that is being experienced in the SLE beluga population. As stated previously, one hypotheses will consider that inbred individuals have a higher rate of mortality from natural causes than do non-inbred individuals. In order to test this hypothesis, the relationship between inbreeding and mortality was assessed. Genetic diversity, in particular, the observed heterozygosity and allelic diversity of the SLE population and Arctic beluga individuals was estimated and compared based on genotyping individuals at 18 microsatellite loci. The goal was to compare the levels of genetic diversity between the SLE population and the Arctic individuals, as the SLE beluga population is

isolated genetically and geographically, where the Arctic belugas are able to migrate across their Arctic waters. It is expected that more inbred individuals will take longer to recover from infections, diseases, pathogens, etc. than do non-inbred individuals. We also expect that DNA markers will reveal low heterozygosity in the small SLE beluga population relative to the more abundant Arctic population. It is also expected that SLE belugas will have a lower genetic diversity than that of arctic populations that are not declining at such rapid rates. To test this, diversity metrics will be used to compare the diversity between the SLE and Arctic beluga populations.

Materials and Methods

Analysis of Extracted DNA

Skin samples from 10 Nunavik Belugas were collected by DFO (Fisheries and Oceans Canada) in 2013. The DNA from these samples was previously extracted by other researchers in the lab as part of their thesis work. However, for my thesis I had to estimate the quantity of DNA in these samples. I did this using spectrophotometry using a NanoDrop 1000 (ThermoFisher Scientific). For each sample, 2 µl of DNA was pipetted on to the NanoDrop, and was compared to three known samples of calf thymus at three different concentrations of 1 ng/µl, 5 ng/µl, as well as 10 ng/µl for reference.

Sex Identification

To assess the quality of the DNA in these samples, I performed a sexing PCR, where I amplified different regions of the X- and Y- chromosomes using the methods described in Gilson et al. (1998). In addition to identifying the sex of each individual, this process also provided

information of how well each sample would amplify in PCR, and therefore indicate which samples had DNA of adequate quality to proceed with microsatellite genotyping. Briefly, these PCR amplifications were conducted in a 20µl reactions which contained 10ng of DNA, 1X PCR buffer (Invitrogen), 0.2 mM each dNTP, 0.4mg/ml BSA, 2 mM $MgCl_2$, 0.5 units/µl *Taq* polymerase (Invitrogen), and 0.3µM of each primer (Y53-3C, Y53-3D, P1-5EZ, and P2-3EZ) found in **Table 1.** The round of samples ran through the PCR also contained a negative control in which 2µl of water was added in lieu of the DNA. The conditions for the PCR cycles were as follows: first, the DNA was denatured for 5 minutes at 94°C. Next, 30 cycles of denaturing, annealing, and extension occurred, with 30 seconds at 94°C, 1 minute at 55°C (annealing temperature), and 1 minute at 72 °C. Finally, a final extension step was performed for 10 minutes at 72°C.

Agarose gel electrophoresis was performed after the PCR amplification to visualize the resulting PCR products. A 1.5% agarose gel was prepared with ethidium bromide (4 µl/100ml) in an 80ml flask. Four microliters of Orange-G loading dye were then added to each sample. This loading dye serves three functions. First, it gives the samples colour, making it easier to visualize the sample during pipetting and gel loading. Second, it makes the samples more dense than the gel buffer, so that the samples sink into the wells of the agarose gels rather than floating away. Third, the Orange-G dye itself migrates through agarose gels at the same rate as a ~50bp fragment of DNA, providing a means to monitor the progress of the gel as it is running. 10 µl of this DNA/Orange-G mixture for each sample was gently pipetted into its own lane on the gel. The gel box was then covered in enough buffer to cover the lanes and the sides of the well. A current was presented to the system at 80 volts, and left to run for about an hour in order to allow the DNA to migrate. Afterwards, the gel was visualized under UV light. A sample that had two

bands indicates that the sample is male, and a sample that had one band indicates that it was a female (**Figure 2, Table 2**).

Multiplex PCR

I genotyped each sample at 18 microsatellite loci that have previously been determined to be variable within the St. Lawrence beluga, and which are currently used routinely for the genetic analysis of this population. Our lab currently genotypes individuals at these loci using a process called "multiplex PCR", where multiple loci are amplified and visualized simultaneously. These multiplex reactions were developed previously by other members of the lab.

The multiplex PCR amplifications of these microsatellite loci were prepared in 10µl reactions. Each tube contained the respective primers and their desired concentrations with respect to **Table 3**, as well as 1x PCR buffer, 0.2mM each dNTP, 0.16mg/ml Bovine Serum Albumin (BSA), 1.5mM $MgCl_2$, 0.05 units/µl *Taq* Polymerase, 2µl (10 ng) of DNA per sample, and H_2O for the remaining amount. One primer of each primer pair used in the multiplex PCR was fluorescently labelled using the following molecules: PET (red), NED (fluoresces yellow, but shows up black on the electropherogram), VIC (green), and 6FAM (blue) (**Figure 1**). Each multiplex reaction performed included 10 samples of Arctic DNA, 1 negative control, and 2 known positive controls (Bel01296, Bel01298).

The 13 samples were amplified at 18 microsatellite loci in each of the 6 multiplexes using methods that were previously outlined in our laboratory (**Table 3**). The PCRs were ran using the following cycling conditions: denaturation of the sample for 5 minutes at 94°C. Next, 30 cycles of denaturing, annealing, and extension occurred, with 30 seconds at 94°C, 1 minute at the

respective annealing temperature (**Table 3**), and 1 minute at 72°C. At last, a final extension step was performed for 45 minutes at 60°C.

Preparation and Scoring of Microsatellite Profiles

The PCR products were then prepared for capillary electrophoresis. The same 13 samples that were amplified in each of the 6 multiplexes were prepared for capillary electrophoresis for a total of 78 samples. 2 µl of PCR product was added to the plate, and water was added to any empty wells. 10µl of formamide/size standard mixture was added to the 2µl sample for each well of the plate.

GeneMarker (v2.7.4) was the program used to score the microsatellite profiles. For each multiplex, all of the size standards were checked and edited manually using the GeneMarker program. The negative controls for each multiplex were also checked for no peaks, as well as checked the positive controls to make sure their scores were correct (**Figure 3**). Allele calls were also edited manually at the 18 loci to ensure correct scoring.

Relatedness and Genetic Diversity

Internal relatedness (IR) for each SLE beluga individual was calculated for 156 individuals for which we had both genotype data as well as a known cause of death. The data for the 156 individuals was input into the R-studio program using the 'ggplot2' package in order to compare the degree of inbreeding to the cause of mortality. The effective population size (N_e) of the SLE beluga population was estimated using the COLONY program (Wang, 2009). Estimates of genetic diversity, specifically the observed heterozygosity and allelic diversity, for the SLE beluga data as well as the 10 Arctic individuals were analyzed using the G-studio program with

the ‘ggplot2’ package in order to compare the observed heterozygosity and the allelic diversity between the two populations (**Figure 5**). Due to the largely differing sample sizes of 789 (SLE beluga individuals) and 10 (Arctic beluga individuals), iterations of 10 for the SLE beluga individuals were sampled randomly 1000 times using G-studio in order to have a more accurate comparison with the Arctic beluga data sample set.

Results

Of the 10 Arctic beluga DNA samples obtained from Nunavik, all were assigned a sex (**Figure 2**). All of the samples had adequate DNA to proceed with microsatellite genotyping. The results of the sexing gel revealed 5 males and 5 females among the samples (**Table 2, Figure 2**).

The relationship between inbreeding (calculated as “internal relatedness”, IR) and cause of death is shown in **Figure 4**. The sample sizes were variable across different causes of death, with certain causes of death showing more inbred individuals than others. However, due to the sample sizes for each cause of death being quite low, no statistical analyses were performed, and this analysis should be interpreted as preliminary.

The estimated effective population size of the SLE beluga was 80 individuals with a 95% confidence interval of 58-112 individuals.

Genetic diversity was substantially lower in the St. Lawrence belugas than in those from the Arctic. Specifically, the distribution of observed heterozygosity calculations based on the SLE individuals, when subsampled to 10 individuals for comparison to the Arctic populations,

was much lower than the heterozygosity value for the Arctic individuals (**Figure 5a**). A similar pattern was found based on the metric of allelic diversity (**Figure 5b**).

Discussion

The goal of the study was to determine the degree to which inbreeding and low genetic diversity are affecting the St. Lawrence beluga population, if at all. By comparing a sample set of Arctic beluga individuals with a sample set of SLE belugas, the hope was to provide insight into the genetic structure of the population of belugas that inhabit the St. Lawrence.

The inbreeding analysis based on the internal-relatedness (IR) of the SLE beluga population shows certain causes of death to be correlated with a stronger degree of inbreeding than others. The observed pattern generally follows my expectations, where there tended to be a relationship between inbreeding and mortality for several natural mortality causes, but no such relationship for human-caused mortalities. However, these results should be taken as preliminary because the sample sizes of genotyped individuals in several mortality categories is still quite low, and it will take many more years of sampling and necropsies to increase these sample sizes and obtain more power for assessing these relationships.

The estimated effective population size for the SLE beluga population was 80 individuals, which is extremely low, particularly given that the population was thought to number just under 1000 individuals during the time the majority of these samples were collected. These data are concerning because an N_e of 80 individuals means that essentially only about 80 individuals are reproducing and passing on their alleles to the next generation of offspring. A low N_e such as this, creates an increased potential for inbreeding within the population, as it determines the degree to which gene frequencies will be transmitted across generations. For

example, the Gulf of California fin whale *(Balaenoptera physalus)* is an isolated population that has remained at a low effective population size of ~360 individuals (Rivera-León et al., 2019). Their low effective population size and high degree of isolation implies that the Gulf of California fin whales are vulnerable to the negative effects of genetic drift, and human-caused mortality (Rivera-León et al., 2019).

Comparison of genetic diversity between the SLE and Arctic population showed substantially lower diversity in the SLE. These results were unsurprising, as the SLE beluga population has been declining for quite some time. A low observed heterozygosity in the SLE beluga indicates little genetic variability within the population. A low allelic diversity for the SLE population is indicative of a limited variety of alleles for the genes within the population, meaning that there are not many genetic differences between individuals. A low allelic diversity can also mean that there are fewer opportunities for the population to adapt in response to environmental changes.

This research is part of an ongoing project that will continue to add more samples to an already existing dataset as more belugas are necropsied over time. However, the results obtained provide preliminary insight into the genetic structure of the SLE beluga population and the level of genetic diversity they possess in comparison to the Arctic population. A larger sample set that includes both genomic and mortality data is crucial for the conservation of the endangered beluga species as a whole (Lesage et al., 2014).

Table 1. Primers used for the determination of sex, as well as DNA quantity and quality of the samples.

Primer	Desired Reaction Concentration (µM)	Ta (°C)	Reference
P1-5EZ	0.3	55	Aasen & Medrano, 1990
P2-3EZ	0.3	55	Aasen & Medrano, 1990
Y53-3C	0.3	55	Gilson et al., 1998
Y53-3D	0.3	55	Gilson et al., 1998

Table 2. Sex of individuals as per the results shown in Figure 2.

Individual	Lane of Gel	Sex of Individual
Bel01342	2	Male
Bel01343	3	Female
Bel01344	4	Female
Bel01345	5	Female
Bel01346	6	Male
Bel01347	7	Female
Bel01348	8	Male
Bel01349	9	Male
Bel01350	10	Male
Bel01351	11	Female
N/A	12	Empty space
N/A	13	Negative control

Table 3. Forward and reverse primers with their fluorescent labels for each multiplex reaction, along with the desired reaction concentration and annealing temperature (Ta).

Primer and Fluorescent Label	Multiplex Reaction	Desired Reaction concentration (µM)	Ta (°C)	Reference
EV37mn-F-VIC	1	0.32	54	Valsecchi & Amos, 1996
EV37mn-R-VIC	1	0.32	54	
FCB5-F-NED	1	0.25	54	Buchanan et al., 1996
FCB5-R-NED	1	0.25	54	
FCB17-F-PET	1	0.15	54	Buchanan et al., 1996
FCB17-R-PET	1	0.15	54	
FCB10-F-6FAM	1	0.2	54	Buchanan et al., 1996
FCB10-R-6FAM	1	0.2	54	
EV14-F-6FAM	3	0.1	52	Valsecchi & Amos, 1996
EV14-R-6FAM	3	0.1	52	
SW19-F-PET	3	0.4	52	Richard et al., 1996
SW19-R-PET	3	0.4	52	
RW48-F-VIC	3	0.35	52	Waldick et al., 1999
RW48-R-VIC	3	0.35	52	
EV94-F-6FAM	4	0.36	56	Valsecchi & Amos, 1996
EV94-R-6FAM	4	0.36	56	
FCB1-F-VIC	4	0.1	56	Buchanan et al., 1996
FCB1-R-VIC	4	0.1	56	
FCB6-F-NED	4	0.5	56	Buchanan et al., 1996
FCB6-R-NED	4	0.5	56	
GATA028-F-NED	5	0.4	52	Honma et al., 1999
GATA028-R-NED	5	0.4	52	
TexVet5-F-NED	5	0.15	52	Rooney et al., 1999
TexVet5-R-NED	5	0.15	52	
MK6-F-VIC	5	0.15	52	Krützen et al., 2001
MK6-R-VIC	5	0.15	52	
TexVet19-F-6FAM	5	0.3	52	Rooney et al., 1999
TexVet19-R-6FAM	5	0.3	52	
FCB14-F-VIC	6	0.4	55	Buchanan et al., 1996
FCB14-R-VIC	6	0.4	55	
RW34-F-6FAM	6	0.09	55	Waldick et al., 1999
RW34-R-6FAM	6	0.09	55	
FCB4-F-PET	8	0.23	58	Buchanan et al., 1996
FCB4-R-PET	8	0.23	58	
FCB3-F-VIC	8	0.62	58	Buchanan et al., 1996
FCB3-R-VIC	8	0.62	58	

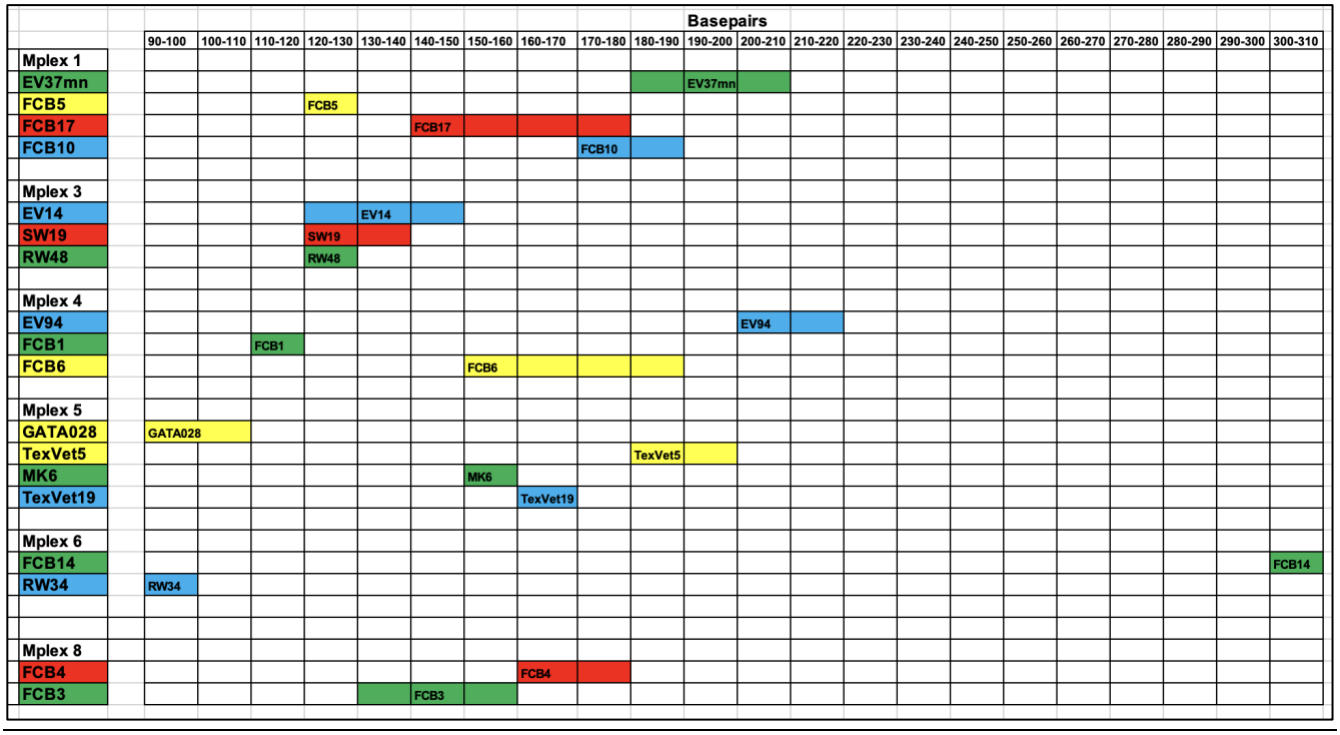

Figure 1. Primer sets used in each multiplex reaction are shown on the left column, as well as their fluorescence and relative lengths in base pairs being shown to the right.

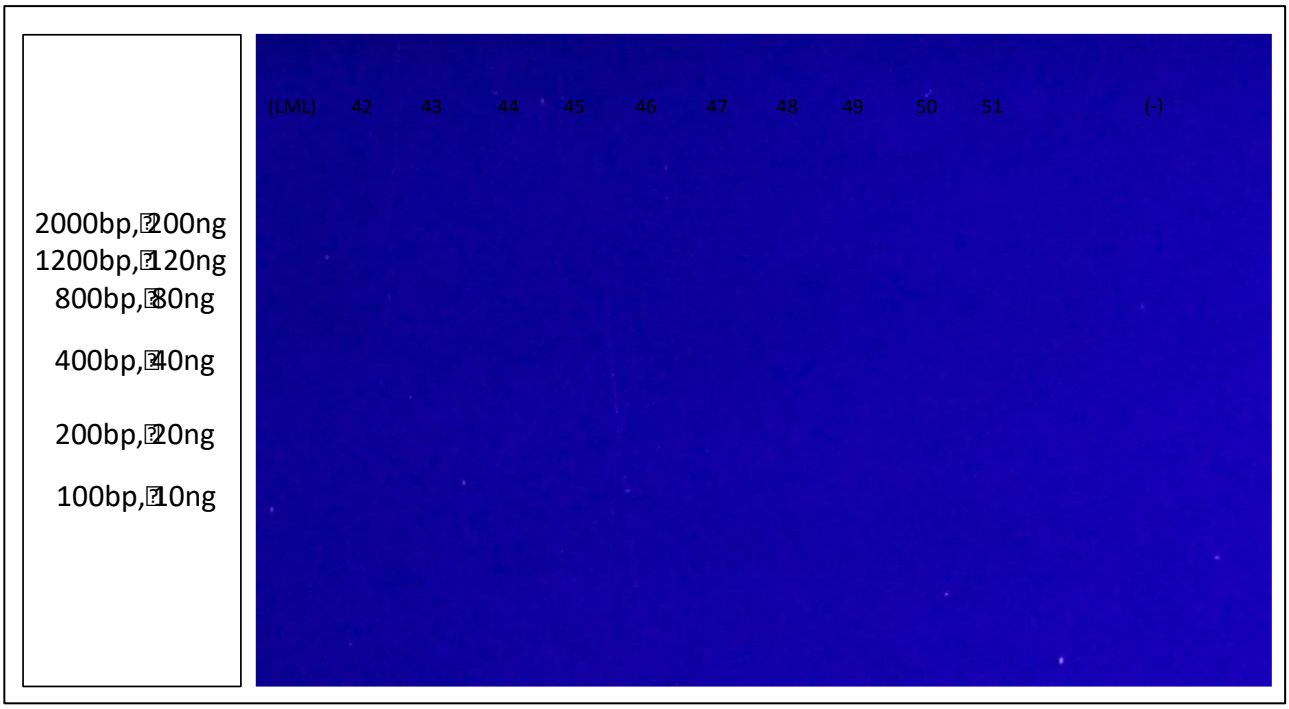

Figure 2. Agarose gel stained with ethidium bromide for the visualization of the sexing PCR product for the determination of sex of the Arctic beluga samples.

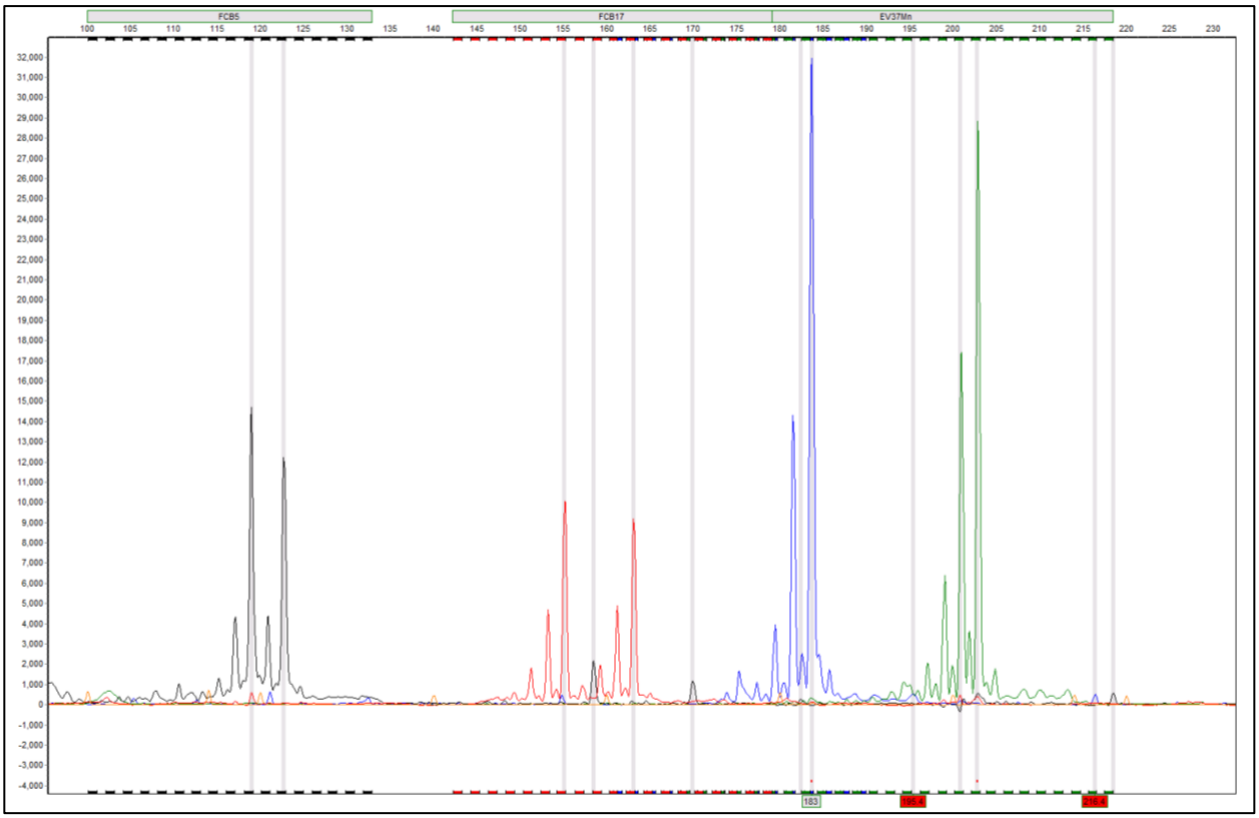

Figure 3. Example electropherogram from Multiplex 1 reaction using the forward and reverse primers EV37mn, FCB5, FCB17, and FCB10.

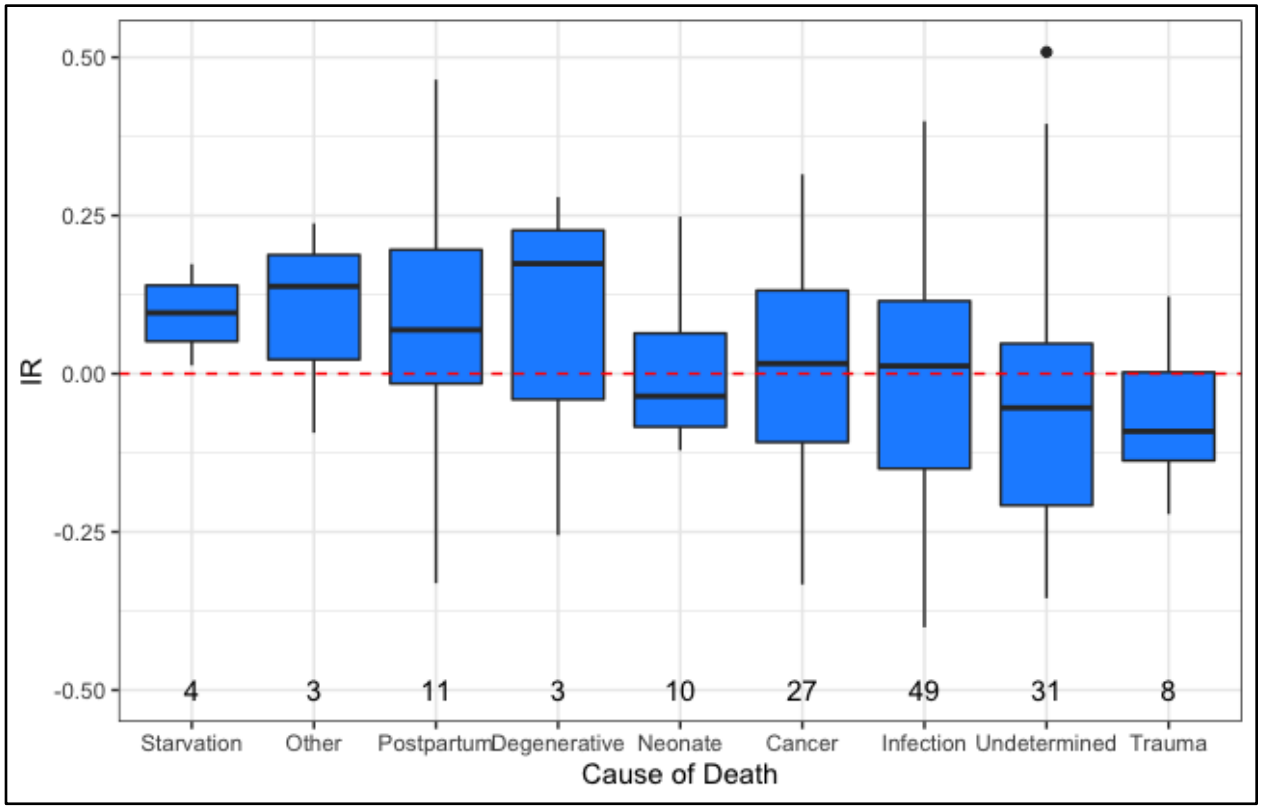

Figure 4. Inbreeding analysis of the SLE beluga data by calculating internal relatedness (IR) with R & R-Studio program showing the relationship between inbreeding and mortality. The numbers above each cause of death, along the x-axis, indicate the sample size of the SLE beluga individuals whose mortality was due to that cause of death.

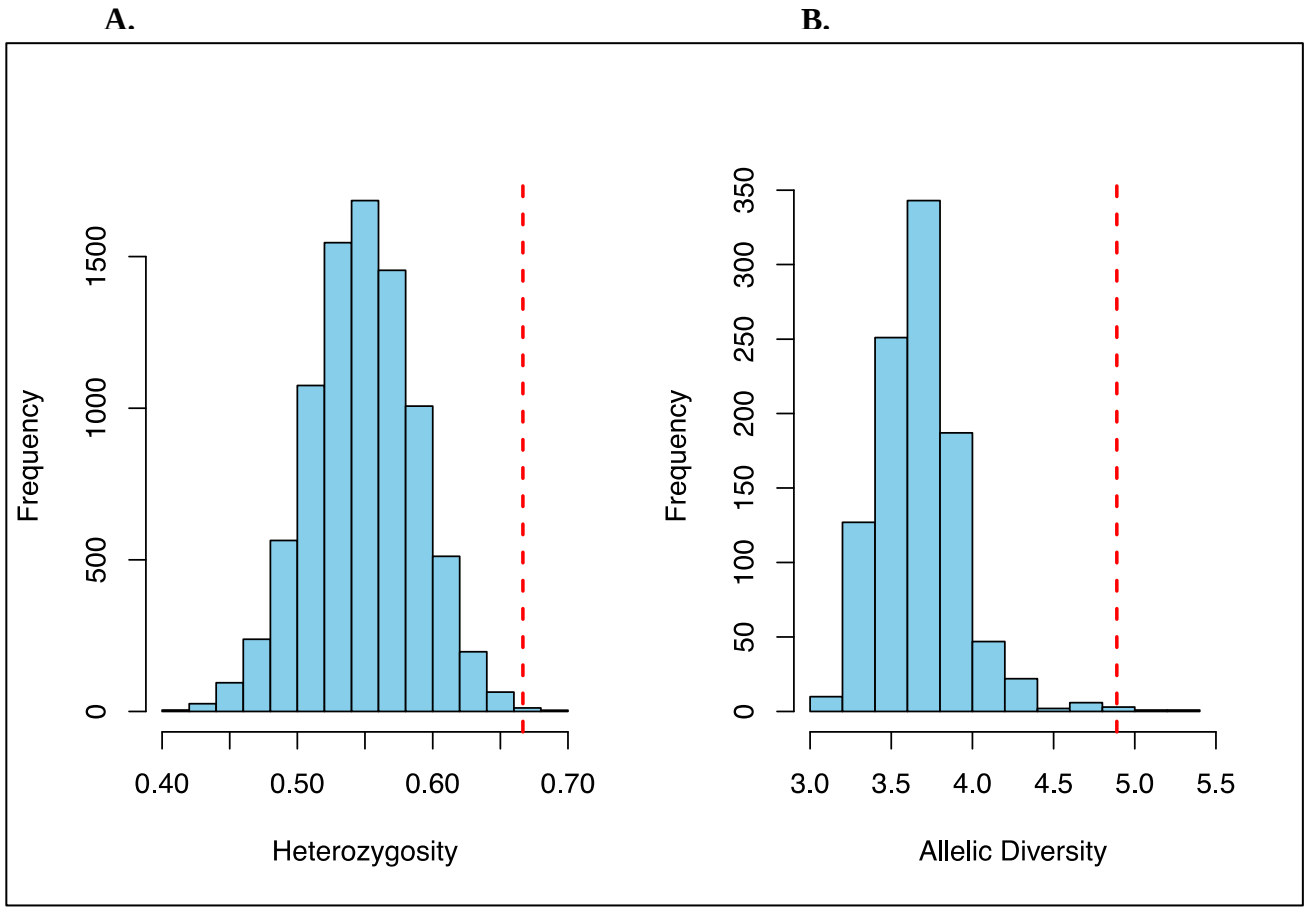

Figure 5. comparison of observed heterozygosity (A.) and allelic diversity (B.) between the SLE beluga data (blue) and Nunavik beluga data (red).

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