

Ringed seal (*Pusa hispida*) population trends
inferred from genetics

By

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Abstract

Ringed seals (*Pusa hispida*) are one of the most common and widely distributed Arctic pinnipeds. They are the primary prey of polar bears (*Ursus maritimus*) and an important food source for northern peoples. Climate change is predicted to affect ringed seals negatively, through the loss of critical breeding habitat. The first objective of my thesis was to assess trends in abundance of ringed seals using genetics. I obtained samples that were collected in the eastern Arctic through Fisheries and Oceans Canada's Community-Based Monitoring program. I extracted and profiled DNA from tissue samples at 14 polymorphic microsatellite loci. I used genetic profiles to estimate the effective number of breeders (N_b) and assessed relationships with counts from aerial surveys (N_s). Only samples that were previously aged were included as they needed to be grouped into birth years to estimate the N_b . I assessed N_b and N_s over time to see if there were temporal trends in either. I did not detect a relationship between N_b and N_s or a temporal trend in either variable when the entire time series was assessed. However, N_b fluctuated over time, with a negative trend from 1983 to 1994 and becoming stable from 1995 to 2012. My second objective was to determine which environmental variables predicted N_b . My analysis suggested that spring rainfall, snow depth, and the date of fall sea-ice freezeup had a greater influence on N_b than the timing of spring breakup. There was a lag of 5 years between N_b and spring rainfall, a 6-year lag between N_b and snow depth, and a 7-year lag between N_b and the date of fall freezeup. In addition to helping create a co-management strategy for ringed seals, gaining an understanding of the impact environmental variables have on ringed seal abundance will be useful in future species at risk assessments. This research helps address the lack of long-term monitoring data available for ringed seals, while improving our understanding of population dynamics of this important Arctic species.

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Thesis Format

This thesis follows the manuscript style, otherwise known as “Sandwich” format as described by the University of Manitoba, Faculty of Graduate Studies. The Introductory chapter gives a general introduction, including background information on the study species and relevant population genetics concepts. Chapter 2 explored the use of effective population size (N_e) as a tool for monitoring ringed seal abundance. In Chapter 3, I compared trends in N_e to environmental variables. I conclude the thesis with a synthesis discussion.

Chapter 1: Thesis introduction

Climate models predict that the warming trends observed over the last several decades will continue and possibly accelerate due to positive feedbacks (Overpeck 1997; Gough & Wolfe 2001). These trends are most pronounced in Arctic regions, where temperatures may increase at two or three times the global average (Comiso 2003, 2012; IPCC 2014). Atmospheric warming has increased ocean temperatures in the Arctic, which has decreased the extent and thickness of sea ice, while causing spring breakup to occur earlier and freezeup later (Gagnon & Gough 2005; Wassmann *et al.* 2011; Lindsay & Schweiger 2015; Wang & Overland 2015). By the middle of the 21st century the Arctic is predicted to be nearly ice free during summer (Overland & Wang 2013).

Ringed seals (*Pusa hispida*) are abundant Arctic pinnipeds that occupy a circumpolar range. They live in close association with sea ice, using it for shelter from predators and the environment (Smith & Lydersen 1991) and as a platform for resting and moulting (Kovacs & Lydersen 2008). The lairs that they construct in snow that accumulates on the pressure ridges of sea ice during winter and spring are important for survival of pups (Smith *et al.* 1991; Kovacs & Lydersen 2008). Ringed seals are at their most vulnerable as pups and are subject to mortality from polar bears (*Ursus maritimus*) (Pilfold *et al.* 2012), Arctic foxes (*Vulpes lagopus*) (Smith 1976), avian predators (Lydersen & Smith 1989), and the environment due to exposure (Smith *et al.* 1991; Stirling & Smith 2004). They are also sensitive to changes in precipitation patterns, including snowfall and rainfall (Stirling & Smith 2004; Ferguson *et al.* 2005; Iacozza & Ferguson 2014).

Ringed seals have been recommended as indicator species to monitor as Arctic conditions change rapidly (Chapman & Walsh 1993; Tynan & Demaster 1997; Laidre *et al.* 2008). Ringed

seals are the primary prey of polar bears (*Ursus maritimus*) and provide food for Arctic foxes (*Vulpes lagopus*) (Stirling & McEwan 1975; Smith 1976; Roth 2003). Northern communities rely on ringed seals as a food source, a source of materials for clothing, and for income (Smith 1987). In recent years, there has been concern that drastic changes in ringed seal abundance may go largely unnoticed by management bodies (Taylor *et al.* 2007). In the southern extent of their range ringed seal body condition has decreased and cortisol has increased indicating that warming conditions may cause stress (Ferguson *et al.* 2017).

Rapid alterations to the sea-ice regime, increased temperatures, and changing precipitation patterns will have negative consequences for many species of Arctic marine mammals that require sea ice for important life history events (Tynan & Demaster 1997; Moore & Huntington 2008; Huntington 2009; Kovacs *et al.* 2011; Laidre *et al.* 2015). Unfortunately, monitoring Arctic marine mammals is difficult (Taylor *et al.* 2007) because of their relative inaccessibility, large distributions, and often cryptic behaviour. Due to the difficulty of studying Arctic marine mammals and the threats posed to them by climate change, pursuing alternative means of monitoring will help to address knowledge gaps, validate monitoring techniques, and inform future conservation efforts.

Ringed seal abundance is difficult to estimate using mark-recapture or aerial surveys. Mark-recapture is challenging because their populations are very large and seals can swim long distances; it is therefore difficult to recapture enough seals to obtain accurate population estimates. For aerial surveys it is difficult to account for the proportion of time that seals spend under snow, ice, and water and therefore cannot be counted (Young *et al.* 2015). The optimal window for surveys to be flown is when the highest proportion of seals are visible, after seals

have left their subnivean lairs, but before the ice breaks up substantially (Kelly *et al.* 2006). However, the date of the optimal window is variable, making it difficult to ensure that surveys are consistent among years (Chambellant *et al.* 2012). Furthermore, the assumption must be made that an equal proportion of seals are hauled out and visible among years that surveys are flown (Kelly *et al.* 2006). Alternative methods of estimating abundance using molecular markers may not be subject to bias that is introduced by the detectability of seals and weather conditions at the time of survey.

Effective population size (N_e) is a central concept in population genetics as it determines the rate of genetic drift and inbreeding, the effectiveness of selection, and the fixation rate of maladaptive mutations (Frankham 1995; Lynch *et al.* 1995). N_e standardizes measures of genetic drift to make populations comparable from a genetic perspective (Hare *et al.* 2011). N_e is the size of an ideal population that will lose genetic variation through drift at the same rate as the actual population being studied (Wright 1931). In the case of N_e , the ideal population is the Fisher-Wright population, which assumes that sex ratios are equal, there is no variation in reproductive success, inbreeding is not significant, generations are not overlapping, population size is constant and finite, and there is no migration or population substructure (Charlesworth 2009). When these assumptions are met, N_e and the census of breeding-aged individuals or census population size (N_c) will be equal. Therefore, the number of breeding individuals can drive trends in N_e . Many of the assumptions of the Fisher-Wright idealized population are typically violated in natural populations, which causes reductions of N_e relative to N_c . For example, in a polygynous population where there are 250 males and 250 females, but only 50 males breed (each with 5 females), the N_c is much higher than the N_e . In this scenario the number of breeding-aged

individuals is 500 and the number of individuals that contribute genetically is 300. However, using the sex ratio estimation method ($N_e = \frac{4 \times N_f \times N_m}{N_f + N_m}$) (Wright 1938) the N_e is 166.67, where N_f and N_m are the number of females and males that breed respectively. The limited number of males breeding causes drift to act on this hypothetical population with the strength that it would in a much smaller ideal population. Therefore, while a variety of factors modify N_e , it is primarily driven by the number of breeding individuals as fewer individuals contributing genetically to the next generation increases the rates of drift and inbreeding (Nunney 2016).

In conservation biology, N_e summarizes the factors (i.e., Wright-Fisher population assumptions) that affect evolutionary processes (Wang *et al.* 2010). While contemporary N_e can be used to monitor evolutionary processes in a population, the effective number of breeders (N_b) (Waples 1989) is typically simpler to quantify in iteroparous species (Waples *et al.* 2013). N_e is measured over a generation while N_b is measured over one reproductive cycle or cohort (Waples 2005; Waples *et al.* 2014; Ferchaud *et al.* 2016). There is a direct theoretical connection between these two parameters as N_b times the generation length is approximately equal to N_e (Waples 1989; Ferchaud *et al.* 2016). The relationship between N_b and N_e varies among taxa in iteroparous species, but most of the variation can be explained by differences in age at maturity and adult life span (Waples *et al.* 2013, 2014). Given that samples can be grouped into birth years, N_b is often more useful for addressing conservation and management questions because the estimates apply to a shorter and more precise time scale than those of N_e (Luikart *et al.* 2010; Tallmon *et al.* 2010; Ferchaud *et al.* 2016). The recent development of single-sample estimators of N_b and N_e (Tallmon *et al.* 2008; Wang 2009; Do *et al.* 2014) has also increased the ability of these parameters to be used in genetic monitoring over shorter time scales (Schwartz *et al.* 2006).

If the ratio between N_e and N_c is generally consistent in ringed seals, I will be able to derive one from the other (Frankham 1995; Schwartz *et al.* 2006; Luikart *et al.* 2010). A recent simulation study shows that N_e closely tracks changes in N_c and virgin biomass (Kuparinen *et al.* 2016). Many studies have been dedicated to characterising the relationships between N_e and N_c in natural populations (Palstra & Fraser 2012; Husemann *et al.* 2016; Yates *et al.* 2017). While several studies have found a direct relationship between N_e and N_c (Côté *et al.* 2013; Kamath *et al.* 2015; Perrier *et al.* 2016; Yates *et al.* 2017), others did not (Whiteley *et al.* 2015; Bernos & Fraser 2016; Mueller *et al.* 2016). Ferchaud *et al.* (2016) and Ruzzante *et al.* (2016) found relationships between N_e and N_c , but suggested that the relationships are complex and require extensive and species/population-specific calibration to design effective monitoring programs. If N_c can be inferred from N_b , this genetic approach could be used as a cost-effective alternative to aerial surveys. Additionally, a stable relationship between the two parameters would allow me to estimate N_c in areas where DNA samples have been collected but aerial surveys have not been conducted.

N_e can be estimated from demographic information (Wright 1938; Kimura & Crow 1963; Nunney & Elam 1994), but complete demographic data is often unavailable for wild populations. In the absence of complete demographic information, N_e can be estimated using genetic markers (Waples 1989; Wang *et al.* 2003; Tallmon *et al.* 2008; Do *et al.* 2014). I used the sibship assignment method (Wang 2009) of estimating N_b from genetic data, as it is the most accurate single-sample method available and accounts for overlapping generations and migration (Wang 2009, 2016) (Figure 2.1). However, many other methods are available for estimating N_e from

genetic data (Wang *et al.* 2003; Hey & Nielsen 2004; Cornuet *et al.* 2008; Nomura 2008; Do *et al.* 2014; Gilbert & Whitlock 2015).

Ancient, historical, and contemporary N_e can all be estimated from genetic data. Ancient N_e encompasses thousands of generations, while historical N_e includes hundreds of generations. Ancient and historical N_e have been used to examine pre- and post- whaling abundances of gray whales (*Eschrichtius robustus*) (Alter *et al.* 2007). Contemporary N_e is more effective for detecting trends that have occurred recently and, therefore, declines that have been caused by rapid climate change (Hare *et al.* 2011). One-sample and two-sample estimators are the two main families of contemporary N_e estimators. Two-sample estimators use changes in allele frequency between two or more sampling events (Wang *et al.* 2003). One-sample estimators require only one sampling event and use either heterozygous excess (Pudovkin *et al.* 1996; Luikart & Cornuet 1999), molecular-coancestry (Nomura 2008), linkage disequilibrium (Waples & Do 2008; Do *et al.* 2014), or sibship assignment (Wang 2009) to estimate N_e . Two-sample estimators are limited by the need to have two samples from a population that are separated by several generations. It is difficult to obtain two samples separated by the 2-4 generations that are required to estimate N_e with precision for long-lived species. The generation time of ringed seals is 11 years (Smith 1973; Palo *et al.* 2001), which would require samples separated by 22-44 years. Additionally, these estimates would apply to the entire time period between the two sampling times. Since these estimates would apply to a large interval of time, it is difficult to examine temporal trends in N_e as calculated with two-sample estimators and to compare those trends to N_c or environmental variables in a way that is biologically meaningful.

Developing genetic methods that can be used to monitor populations is important, as for some species it will be easier to collect genetic data over large spatial and temporal scales. The exploration of genetic methods for species monitoring will be helpful for determining situations where they offer advantages over other methods or where further development is needed. My thesis applies a genetic approach to monitoring ringed seals in the Eastern Canadian Arctic with the intent to provide monitoring options to co-managers. In Chapter 2, a method for inferring temporal trends in abundance from genetic data is developed and tested. In Chapter 3, N_b estimates are generated for a multi-decadal time series and compared to environmental variables that influence recruitment. My analyses explore the relationships between N_b and spring rainfall, snow depth, date of spring breakup, and date of fall freezeup. By examining relationships between population size of ringed seals and environmental variables in the southern extent of their range, I hope to gain an understanding of how ringed seal populations will respond as the effects of climate change become more pronounced throughout the Arctic.

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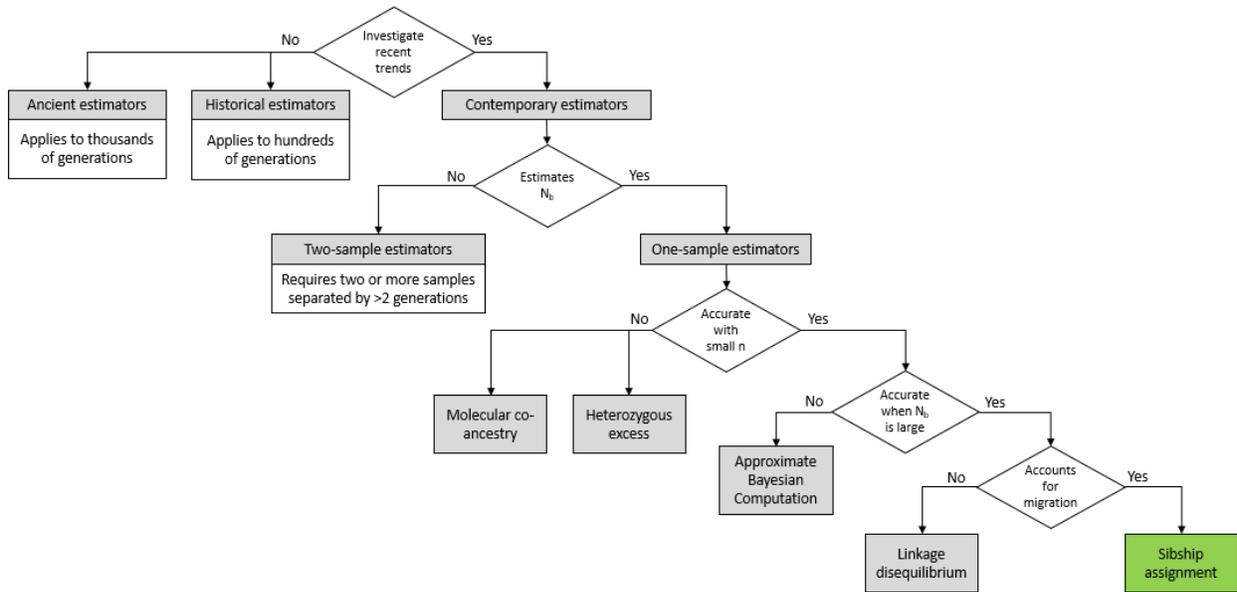


Figure 1.1 Decision tree used to evaluate the most appropriate method to estimate the effective number of breeders (N_b) in ringed seals. The final decision of estimator used is depicted in green.

Chapter 2: Effective number of breeders and the relationship with aerial survey counts in a widely-distributed Arctic seal

Abstract

Ringed seals (*Pusa hispida*) have a circumpolar distribution and use subnivean lairs on sea ice to rear young in spring. In Hudson Bay, the date of spring breakup has advanced by 5.5 days per decade over the past three decades. Earlier breakup of sea ice may compromise the protection from predators and the harsh environment that these lairs provide, which may increase juvenile mortality and cause population decline. However, earlier breakup of sea ice also makes it more difficult to time aerial surveys with peak haul-out of ringed seals, making it difficult to ensure consistency between survey years. Our objective was to estimate trends in population size of ringed seals using genetic methods and compare those estimates with population estimates from aerial surveys (N_s), to see if the aerial surveys estimates are accurate. We used N_s estimates from aerial surveys previously conducted in northwestern Hudson Bay and calculated the parameter effective number of breeders (N_b), defined as number of breeding-aged individuals in the population, using the sibship assignment method. There was no significant trend in the density of ringed seals over time based on estimates from aerial surveys. However, N_b declined in the late 1980s and early 1990s and remained stable in the late 1990s and 2000s. We did not detect a stable positive relationship between N_b and N_s , which suggests that one or both survey monitoring techniques may be biased and that the relationship between these two parameters is complex. Our results suggest that aerial surveys are not an effective method for monitoring ringed seal abundance. Given the importance of ringed seals to food security in the north and as the primary prey of polar bears, developing methods to monitor ringed seals is crucial.

Introduction

In recent years the Arctic has warmed faster than any other biome on earth (Serreze *et al.* 2008; IPCC 2014), which has driven changes to habitat conditions (Jenssen 2006; Stroeve *et al.* 2012; Lindsay & Schweiger 2015) that will threaten several Arctic marine mammal species (Laidre *et al.* 2008, 2015; Kovacs *et al.* 2011; Gilg *et al.* 2012). As the effects of climate change become more pronounced, monitoring abundance will be essential for designating species as conservation concerns, implementing management practices, and providing legal protection. Ice-adapted seals, such as ringed seals (*Pusa hispida*), have been suggested as indicator species to monitor as Arctic conditions change (Chapman & Walsh 1993; Tynan & Demaster 1997; Laidre *et al.* 2008; Maslanik *et al.* 2011). However, estimating population size is difficult for ringed seals, as they occupy expansive and continuous ranges, occur in relatively inaccessible areas, and have cryptic behaviour (Laidre *et al.* 2015).

Current abundance estimates for ringed seals are poor and outdated, with existing estimates restricted to relatively small spatial scales (Laidre *et al.* 2015). Capture-mark-recapture has been unsuccessful when attempted because the recapture probability is very low (S. Ferguson, personal communication). More typically, aerial surveys are used to estimate ringed seal abundance; however, percentage of seals not available to be counted at the surface is often unknown and can vary considerably due to environmental conditions (Smith & Hammill 1981; Frost *et al.* 2004; Krafft *et al.* 2006). Aerial surveys are conducted in the spring when most ringed seals haul-out on the ice to moult (Kelly *et al.* 2010). Seals spend more time hauled-out on sea ice during spring (Carlens *et al.* 2006), presumably because warmer temperatures promote skin growth and accelerate the moulting process (Feltz & Fay 1966). Unfortunately, it is

difficult to time surveys with peak haul-out (Chambellant *et al.* 2012a) and with breakup tending to occur earlier, the optimal time to conduct a survey may no longer coincide with peak haul-out (Lunn *et al.* 1997).

To estimate population size and trends, other methods that do not rely on visual observations have been explored (Boulanger *et al.* 2008; Luikart *et al.* 2010; Côté *et al.* 2013; Conn *et al.* 2014), such as the use of genetic samples for population monitoring. Effective population size N_e determines the rate at which genetic drift will occur, where genetic drift is the rate that allele frequencies will change in a population over time in the absence of selection (Wright 1931, 1938). Smaller populations have lower N_e , which results in more rapid genetic drift (Kliman *et al.* 2008). N_e serves as a convenient way of summarizing evolutionary processes that act on a population, and standardizes them by producing a value that represents the size of an idealized population where drift would be occurring at the same rate as it is in the population of interest (Wright 1938; Wang *et al.* 2010). This metric has been used widely for estimating the extinction and inbreeding depression risks in small populations (Newman & Pilson 1997; Jamieson & Allendorf 2012; Frankham *et al.* 2014). Recently, N_e has been used to monitor trends in abundance and infer population size. Several studies have demonstrated that N_e can be a useful proxy for the number of breeders within a population (Côté *et al.* 2013; Bernos & Fraser 2016; Hall 2016). The effective number of breeders (N_b), which is easier to estimate, is often used in place of N_e (Waples & Antao 2014). N_b is the effective population size during one reproductive event opposed to over a generation that N_e applies to (Waples 2005; Waples *et al.* 2014; Ferchaud *et al.* 2016). N_b allows researchers to gain an understanding of eco-evolutionary

dynamics in iteroparous species with overlapping generations while providing a direct link to standard population genetics theory derived for N_e (Waples *et al.* 2013; Waples & Antao 2014).

Inferring abundance from estimates of N_e relies on knowing the relationship between N_e and census population size (N_c), where the ratio between them is assumed to be constant (Luikart *et al.* 2010; Tallmon *et al.* 2010; Yates *et al.* 2017). When the assumptions of the Wright-Fisher idealized population are violated, N_e is reduced with respect to N_c . These assumptions are that sex ratios are equal, the population has a finite and constant size, equal reproductive success between individuals, generations do not overlap, and there is no genetic or spatial structure (Charlesworth 2009). The average N_e/N_c ratio from meta-analyses of studies using fishes, amphibians, and insects has been calculated as 0.231, and the ratio of N_b to N_c as 0.255 (Palstra & Fraser 2012).

The definition of N_c in population genetics differs from its definition in common usage. Generally, N_c is defined as the total number of individuals in a population. In studies that compare N_e to N_c , it is most commonly defined as the number of breeding-aged individuals (ex. Bernos *et al.* 2017). Often, aerial surveys are unable to distinguish between adults and subadults in seal species (Curtis *et al.* 2011). As the counts from aerial surveys for seals do not include all the individuals in a population or differentiate between adults and subadults we refer to the counts from surveys as N_s .

Our objectives were to estimate the N_e/N_s ratio in ringed seals and to test for a relationship between the two parameters to determine if N_e is indicative of ringed seal abundance. We explored the use of currently available genetic N_e estimators to (1) assess temporal trends in N_e and (2) compare estimates of N_e to estimates of N_s .

Methods

Study area and samples

We derived estimates of N_s by multiplying estimates of ringed seal density from aerial surveys (Chambellant *et al.* 2012a; Young *et al.* 2015) conducted in western Hudson Bay by the size of the area ringed seal samples were collected from for genetic analysis (Figure 2.1). Transects were flown perpendicular to the coast, between Churchill, Manitoba and Arviat, Nunavut (Chambellant *et al.* 2012a; Young *et al.* 2015). Density of ringed seals was estimated with the equation $\hat{D} = \sum_{i=1}^k n_i / (w \sum_{i=1}^k l_i)$ (Buckland *et al.* 2004), where \hat{D} denotes density (km^2), k the number of transects flown, n the number of ringed seals counted, i the respective transect, w the transect width, and l the transect length. Ringed seal samples were collected by subsistence hunters from Arviat, Nunavut. Samples consisted of tissue for DNA extraction and lower jaws from which a tooth was extracted for aging. As specific location data were rarely associated with ringed seal samples from Arviat, we used harvest information for beluga (*Delphinapterus leucas*) in Arviat to estimate the size of the area that subsistence hunters typically harvested from. We assumed that hunters use approximately the same area to hunt both ringed seals and beluga. Harvest data was obtained from the Nunavut Wildlife Management Board (www.nwmb.com/en/publications/harvest-study) and was collected as part of the Nunavut Wildlife Harvest Study (Priest & Usher 2004). We used beluga harvest locations to create a minimum convex polygon then estimated the area enclosed in the polygon with the package *rhr* (Signer & Balkenhol 2015) in R. The resulting area was multiplied by ringed seal density to estimate N_c .

DNA extraction and genotyping

Genomic DNA was extracted from all samples using a Thermo Scientific GeneJET Genomic Purification Kit® (Thermo Scientific, Nashville, USA). We followed the manufacturer's protocol apart from omitting the addition of RNase. A NanoVue™ Plus Spectrophotometer (GE Healthcare, USA) was then used to quantify the DNA in each sample. We used polymerase chain reaction (PCR) to amplify fluorescently labelled microsatellite loci. PCR recipes are listed in Appendix A (Table A1.). PCR runs were performed on a C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario, Canada). Once optimal temperatures were determined for each primer, they were arranged into five multiplex reactions (Appendix A: Table A2.). PCR runs consisted of an initial denaturing step at 95°C for 5 minutes, followed by 32 cycles with a 5-minute 95°C denaturing step, a 1-minute annealing step at the temperatures listed above in respective reactions, and a 45-second DNA synthesis step at 72°C. These cycles were followed by a 30-minute extension step at 72°C. PCR products were visualized using an ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems, USA). The microsatellite data that we obtained was merged with data from Petersen (2008). Extraction and amplification protocols for pre-existing microsatellite data can be found in Petersen (2008).

The loci amplified were: Hg4.2, Hg6.1, Hg6.3, Hg8.10, and Hg8.9 (Allen *et al.* 1995); Hi15, Hi16, Hi2, Hi8, Lc28, and Lc6 (Davis *et al.* 2002); and SGPv10, SGPv11, and SGPv9 (Goodman 1997) (Appendix A: Table A3.). These dinucleotide primers were previously developed for grey seal (*Halichoerus grypus*), European harbour seal (*Phoca vitulina vitulina*), Weddell seal (*Leptonychotes weddellii*), leopard seal (*Hydrurga leptonyx*), and crabeater seal

(*Lobodon carcinophagus*). They have all been shown to cross-amplify in ringed seal (Davis *et al.* 2002; Petersen 2008).

Microsatellite analysis

Presence of homozygote excess, null alleles, large allele dropout, and stutter in the data set were examined in MICRO-CHECKER (Van Oosterhout *et al.* 2004). We used the program PEDANT (Johnson & Haydon 2007), which implements a maximum-likelihood approach to quantify null alleles and long allele dropout rates. Genotyping error rates were required by GENECLASS2 (Piry *et al.* 2004), which we used to remove first generation migrants that may confound N_b estimates produced with COLONY2 (Jones & Wang 2010), which also required null allele and long allele dropout rates. GENEPOP v 4.2.2 (Raymond & Rousset 1995; Rousset 2008) was used to test for deviations from Hardy-Weinberg equilibrium (HWE). To account for multiple pairwise comparisons in HWE and LD, we adjusted α using Holm's sequential Bonferroni correction (Holm 1979; Rice 1989) with an α of 0.05 .

COLONY2 requires genotypic data from a sample of individuals born in the same reproductive cycle to estimate N_b . To sort samples into birth years, we subtracted their age from the year they were collected. Samples were aged using the dentine and cementum methods (Smith 1973; Stewart *et al.* 1996). When N_b is estimated with genetic data there are quantitative bias corrections that should be applied. For this reason, estimates of N_b and N_e obtained from genetic data, before bias corrections have been applied, are referred to as raw/unadjusted estimates and are denoted as $N_{b_{raw}}$ and $N_{e_{raw}}$. By sorting samples into cohorts based on the reproductive event or breeding season that gave rise to them, single-sample estimators can be used to estimate N_b , rather than N_e . This helps to eliminate the problem of overlapping

generations, which may bias estimates. Therefore, sibship assignment (SA) as implemented through the program COLONY2 (Jones & Wang 2010) was chosen as the most appropriate method for estimating the N_b of ringed seals. The sibship assignment method assumes that as sibling frequency increases as N_b decreases (Bernos *et al.* 2017). Methods that use molecular coancestry (Nomura 2008) and heterozygous excess are highly biased when applied to large populations (Wang 2016). The linkage disequilibrium method available in NEESTIMATOR v2 (Do *et al.* 2014) estimates N_b accurately when adequate sample sizes and informative markers are available. However, it does not account for migration and is more appropriate for closed populations (Gilbert & Whitlock 2015). It can still provide accurate estimates of local N_b or N_e when migration levels are below ~5-10% (England *et al.* 2010). Estimates from the LD method can apply to either one or a few previous generations, if residual disequilibria have not yet have been broken down through recombination (Waples 2005; Wang 2016). The SA method applies only to the parental generation and accounts for migration if first-generation migrants are identified and removed (Wang 2009, 2016). When the sample size is small relative to the actual N_b , SA tends to underestimate N_b while LD tends to overestimate N_b (Wang 2009, 2016).

COLONY2 is a more appropriate N_b estimator than NEESTIMATOR v2 because, in addition to accounting for migration, it allows the user to specify different breeding systems for males and females. Furthermore, with small sample sizes, estimates of N_b from COLONY2 contain upper confidence intervals that span to infinity less often than NEESTIMATOR v2. Female ringed seals typically only have one offspring in a year (Spotte 1982), while males can sire several (Krafft *et al.* 2007); therefore, female monogamy and male polygyny were selected when running COLONY2. As recommended, the analysis method used was full-likelihood, non-random mating

was assumed, and inbreeding was not selected (Jones & Wang 2010). Inbreeding does not appear to be significant in Hudson Bay ringed seals (Petersen 2008). No prior N_e estimates were used, and the error rates estimated in PEDANT were included. Although some estimates of N_b contained upper confidence intervals that spanned infinity, all mean estimates were retained as a previously conducted sensitivity analysis with COLONY2 indicated that even when $n \leq 20$, robust estimates of N_b were obtained (Ozerov *et al.* 2015).

We used the quantitative bias corrections derived by Waples *et al.* (2014) to account for bias in N_b estimates and to estimate N_e . These corrections require adult lifespan (AL) to be computed with the formula $AL = \omega - \alpha + 1$ (Nunney & Elam 1994), where α = mean age at maturity and ω = maximum age. To estimate unbiased N_b the following equation from Waples *et al.* (2014) was used:

$$N_b = \frac{N_{b_{raw}}}{1.103 - 0.245 \times \log(AL/\alpha)}$$

To estimate N_e we used the following equation from Waples *et al.* 2014:

$$N_e = \frac{N_b}{0.485 + 0.758 \times \log(AL/\alpha)}$$

α of female ringed seals was determined by examination of the ovaries for presence of corpus luteum or a fetus (Chambellant *et al.* 2012b). Age at maturity of female ringed seals in western Hudson Bay has declined from 5.4 years in the 1990s to 3.8 in the 2000s. Therefore, when calculating AL we used $\alpha = 5.4$ for years preceding the year 2000 and $\alpha = 3.8$ for 2000 and subsequent years (Chambellant *et al.* 2012b). Age at maturity has not been calculated for males, but evidence suggests that they do not become sexually mature until age 7 (Breton-Provencher).

Since males compete for mates with other males, older larger males likely have greater success in acquiring mates, meaning that they breed more when they are older. Since males contribute genetically to the population more when they are older, we used an $\alpha = 8.0$ for years preceding the year 2000 and subtracted 1.6 for years 2000 and onward. We assumed that the same reduction in age at maturity observed in females. We added the average of male α and female α to use in the corrections. A ω of 37 was used for years preceding 2000 and ω of 33 for subsequent years (Chambellant *et al.* 2012b).

Statistical analysis

We used generalized linear models (GLMs) with a gamma distribution and an inverse link function to test for temporal trends in the entire series of N_b estimates and to test for temporal trends in N_b , during and prior to the time aerial survey estimates were available (1995-2013). Ratios of N_b/N_s were calculated using N_b and N_s estimates. Although samples used to estimate N_b were from cohorts 1981 to 2012, N_b estimates apply to the parental generation of the sample, resulting in a one-year lag in N_b relative to N_s . The N_b/N_s ratio was calculated for all years where a pairing of N_b and N_s where both estimates were available. A pooled ratio was calculated by taking the harmonic mean of N_b/N_s and N_e/N_s from all years (Motro & Thomson 1982).

Results

MICRO-CHECKER indicated that stutter may have been present at Hg6.1, which was omitted from further analysis (Appendix A: Table A4.). No loci deviated significantly from HWE after Holm's Bonferroni corrections (Appendix A: Table A5.). Observed, expected, and unbiased expected heterozygosity were all high and relatively stable over time (Table 2.1).

Genotyping error rates are listed in Appendix A (Table A8.). GENECLASS2 did not detect any first-generation migrants. The harmonic mean of COLONY2 N_b estimates was 76 and ranged from 44 to 213 (Table 2.2). The estimate of N_b for 2009 was omitted from further analyses because it was substantially higher than all other estimates, likely due to a rare allele that can bias estimates, especially when sample sizes are small (Waples & England 2011). Sample sizes of less than 20 often included confidence intervals that spanned infinity; however, we used all estimates that were not identified as outliers during statistical downstream analyses. The size of the MCP that enclosed all points where belugas were harvested in the Arviat area was 1711.6 km^2 .

The regression of N_b over time indicated no significant trend in N_b ($R^2 = 0.077$, $T_{24} = -1.27$, $p = 0.218$) (Figure 2.2A). Additionally, partial regressions of N_b , before and during the time aerial surveys were conducted, indicated a decline from 1983-1994 ($R^2 = 0.539$, $T_{10} = -3.52$, $p = 0.006$) before aerial surveys were flown, but during the time surveys were flown, N_b was stable ($R^2 = 0.184$, $T_{13} = 1.70$, $p = 0.115$) (Figure 2.2).

N_s estimates ranged from 335.5 in 2013 to 2086.5 in 1995 (Table 2.2). N_s did not change significantly over time ($R^2 = 0.368$, $T_8 = -2.12$, $p = 0.067$) (Figure 2.2B). The pooled ratio of N_b/N_s was 0.048 and $N_{e(Adj)}/N_s$ was 0.060 (Table 2.2). We did not find a significant relationship between N_b and N_s ($R^2 = 0.543$, $F_5 = 2.04$, $p = 0.097$) (Figure 2.3).

Discussion

Over the length of our study there was a significant decline in N_b until 1994, followed by stable N_b from 1995 to 2011. The decline in N_b in the 1990s corresponds to lower recruitment of ringed seals in western Hudson Bay in the 1990s, which was attributed to less snowfall and

lower snow depth in western Hudson Bay (Ferguson *et al.* 2005). We did not observe a significant, positive relationship between N_s estimates from aerial surveys and our genetic estimates of N_b , as we had expected. The lack of a relationship between N_b and N_s is concerning, because aerial surveys are the most common method used to monitor ringed seal density and distribution, and if they are inaccurate than we may not be able to detect a decline in ringed seals and take appropriate conservation actions.

The decline in N_b in the late 1980s and early 1990s is similar to N_e estimates for endangered Saimaa ringed seal (*Phoca hispida saimensis*), where N_e was lower in the 1980s and 1990s, and higher in the 2000s (Valtonen *et al.* 2014). Temperatures during winter were especially cold in both locations during the late 1980s and early 1990s (Tietäväinen *et al.* 2010; Chambellant *et al.* 2012). In western Hudson Bay, pregnancy rates and pup survival were low in the 1990s (Ferguson *et al.* 2005; Stirling 2005) and increased in the 2000s (Chambellant *et al.* 2012b). During the early 1990s, when N_e estimates were lowest, ‘heavy’ ice conditions were noted in western Hudson Bay, including late date of breakup, short open-water periods, cold temperatures, and high ice cover in late spring (Chambellant *et al.* 2012). In Finland snow-ice thickness was higher in the 1980s and 1990s, but decreased after the year 2000 (Korhonen 2006). Heavy-ice conditions have been shown to negatively affect reproductive success of ringed seals in the western Arctic (Smith 1987; Stirling 2002; Nguyen *et al.* 2017). An additional factor that may have linked the environmental conditions of many regions in the 1990s was the 1991 eruption of Mount Pinatubo, which released an aerosol cloud that affected the Arctic Oscillation (Stenchikov *et al.* 2002) and reduced air temperatures in the Northern Hemisphere (Parker *et al.* 1996).

The estimates of N_s from aerial surveys and of N_b indicated contrasting temporal patterns from 1995 to 2013, although neither trend was significantly different from zero. Although there was not a significant trend in N_s the highest estimate was from the first survey in 1995 and the lowest estimate was from the most recent survey in 2013. The negative slope in N_s ($p = 0.067$) would likely lead to much different inferences about ringed seal population trends than analysis of N_b during this time.

Our analysis of the relationship between N_b and N_s was limited to 6 years that aerial surveys were flown and tissue samples were available for estimating N_b . Since we did not observe a consistent relationship between N_b and N_s in our study area, we were unable to calibrate the two monitoring techniques. As these two monitoring techniques are difficult to apply to this changing ecosystem, and both have potential biases and sources of error, one or both may not accurately reflect population trends. There are many sources of error when using aerial surveys to estimate abundance or density of ice dwelling seals (Young *et al.* 2015). Variability of environmental factors, including wind conditions and precipitation, can cause animals to be missed and bias counts from aerial surveys downward (Caughley 1974; Born *et al.* 1999; Young *et al.* 2015). The greater concern with aerial surveys for long-term monitoring is consistency among years. While spring breakup trends towards occurring earlier in western Hudson Bay (Lunn *et al.* 2016) it may be harder to time surveys to coincide with peak moulting, when the most seals are hauled out and can be counted. N_b may be influenced by other demographic or abiotic factors, which warrants further exploration to determine what drives N_b in Hudson Bay ringed seals. The absence of a relationship between N_b and population size has previously been noted in some studies (Whiteley *et al.* 2015; Mueller *et al.* 2016).

Our estimated ratios of $N_b/N_s = 0.048$ and $N_{e(Adj)}/N_s = 0.060$, were similar to previously published N_e/N_c ratios that used single-sample N_e estimators, in *Pusa hispida saimensis* (Valtonen *et al.* 2014), *Phocarctos hookeri* (Collins *et al.* 2016), and *Phoca vitulina* (Andersen *et al.* 2011) (Table 2.3). Our estimates were also similar to those for *Arctocephalus australis* (de Oliveira *et al.* 2006) when the sex ratio equation (Wright 1931, 1938) was used to estimate N_e . However, the N_e/N_c ratio for *Pusa hispida saimensis* (Valtonen *et al.* 2014), when estimated with the contemporary temporal-method, was higher (0.36) than our estimated ratios. They attributed the differences in N_e estimates from single-sample and temporal estimators to population structure biasing single-sample estimators downward. The temporal method when implemented through MLNE (Wang *et al.* 2003) accounts for population substructure, whereas single-sample estimators have been shown to be biased downward when sampling includes multiple genetic neighbourhoods (Neel *et al.* 2013).

Population genetics theory and empirical studies predict that the ratio of N_e to N_c will be 0.1 to 0.5 (Nunney 1991, 1993, 1996; Nunney & Elam 1994; Frankham 1995; Palstra & Fraser 2012; Waples 2016). All our estimated ratios fell below 0.1, which may have been caused by our use of N_b to N_s rather than N_e and N_c . Another potential source of error that we could not account for was young-of-the-year seals being included in N_s . Ideally N_s would include only breeding-aged seals. In our study, a large proportion of the sample from aerial surveys likely consisted of young-of-the-year seals, which would inflate the estimate of N_s and thereby reduce the ratio of N_b to N_s .

Although population size affects N_b , we have not investigated the effects of other population parameters that can influence N_b . The effect of reproductive success on N_b may be an

important predictor. In species that have high juvenile mortality, reproductive success, rather than N_c , can have a bigger role in determining the N_e of a population (Nunney 1991; Lee *et al.* 2011). Additionally, environmental variables may also have an impact on N_b . For example, Whiteley *et al.* (2015) found that streamflow, rather than N_c constrained N_b in brook trout. Given the importance of ice in the life cycle of ringed seals, which rely on it for shelter, rearing young, and moulting, further analysis of the relationship between N_b and environmental variables may provide valuable insights into ringed seal population dynamics.

Ringed seals are difficult to monitor, and timing aerial surveys to coincide with peak haul-out will likely become increasingly challenging with the loss of spring sea ice. Therefore, it is crucial to develop and maintain monitoring strategies for this species in order to successfully manage them (Laidre *et al.* 2015). New methods for monitoring ringed seal abundance should be further explored, especially as molecular analysis becomes increasingly affordable and techniques for estimating N_e and other genetic parameters continue to improve. Given the importance of ringed seals and the profound climatic changes occurring in the arctic, developing systems to monitor them that management decisions can be made from is crucial.

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Table 2.1 Sample size (n), mean number of alleles (N_a), expected heterozygosity (H_e), observed heterozygosity (H_o), inbreeding coefficient (F_{IS}), for each year tissue samples from Arviat were available.

Year	n	N_a	H_e	H_o	F_{is}
1980	5	5.000	0.696	0.791	-0.136
1981	5	5.077	0.737	0.855	-0.160
1982	6	6.692	0.767	0.856	-0.116
1983	10	7.923	0.787	0.827	-0.054
1984	9	7.769	0.807	0.831	-0.033
1985	7	7.615	0.804	0.888	-0.112
1986	18	10.61	0.837	0.861	-0.027
1987	18	10.53	0.827	0.874	-0.059
1988	19	11.15	0.829	0.834	-0.007
1989	14	9.462	0.813	0.829	-0.023
1990	7	7.077	0.795	0.872	-0.099
1991	17	9.615	0.807	0.840	-0.049
1992	15	8.692	0.809	0.799	0.012
1993	15	9.846	0.811	0.829	-0.026
1994	11	8.923	0.800	0.864	-0.075
1995	6	5.462	0.731	0.844	-0.142
1996	12	8.385	0.804	0.842	-0.043
1997	7	7.077	0.769	0.830	-0.082
1998	10	7.385	0.794	0.838	-0.055
1999	12	9.385	0.818	0.862	-0.057
2000	32	12.692	0.842	0.852	-0.013
2001	33	12.154	0.832	0.840	-0.007
2002	45	13.462	0.838	0.825	0.018
2003	32	12.846	0.827	0.804	0.029
2004	40	13.538	0.833	0.820	0.012
2005	16	8.846	0.801	0.795	0.009
2006	13	9.231	0.802	0.824	-0.021
2007	25	11.462	0.834	0.840	-0.002
2008	18	10.692	0.832	0.879	-0.060
2009	12	7.077	0.669	0.632	0.055
2010	3	3.769	0.663	0.846	-0.307
2011	11	8.538	0.802	0.832	-0.040

Table 2.2 The effective number of breeders before correction for adult lifespan ($N_{b_{raw}}$) and 95% confidence intervals (CI) were estimated with the program COLONY2. The counts from aerial surveys (N_s) were estimated by multiplying the density of ringed seals from each aerial surveys (Chambellant *et al.* 2012a; Young *et al.* 2015) by the approximated seal harvesting area (1711.6 km^2). The adjusted effective number of breeders (N_b) and adjusted effective population size (N_e) were obtained using the corrections for life history traits (Waples *et al.* 2013). Estimates are listed in the year that they applied to.

Year	$N_{b_{raw}}$	95% CI of N_b	N_s	$N_{b_{raw}}/N_s$	N_b	N_b/N_s	N_e	N_e/N_s
1983	205	67-∞	-	-	213	-	297	-
1984	158	53-∞	-	-	164	-	229	-
1985	82	27-∞	-	-	85	-	119	-
1986	47	25-121	-	-	49	-	68	-
1987	111	55-504	-	-	115	-	161	-
1988	114	57-623	-	-	119	-	165	-
1989	57	28-305	-	-	59	-	83	-
1990	120	33-∞	-	-	125	-	174	-
1991	64	33-205	-	-	67	-	93	-
1992	42	20-185	-	-	44	-	61	-
1993	62	30-914	-	-	64	-	90	-
1994	47	21-405	-	-	49	-	68	-
1995	71	24-∞	2086	0.034	74	0.035	103	0.049
1996	44	20-798	1698	0.026	46	0.027	64	0.038
1997	∞	1-∞	1159	-	∞	-	∞	-
1998	62	27-∞	-	-	64	-	90	-
1999	97	41-∞	762	0.127	101	0.132	141	0.185
2000	66	42-112	1037	0.064	72	0.069	77	0.074
2001	56	35-91	-	-	61	-	65	-
2002	94	62-150	-	-	102	-	109	-
2003	101	63-193	-	-	110	-	117	-
2004	80	52-129	-	-	87	-	93	-
2005	75	34-602	-	-	82	-	87	-
2006	92	41-2630	-	-	100	-	107	-
2007	54	31-99	1570	0.034	59	0.037	63	0.04
2008	67	37-216	751	0.089	73	0.097	78	0.104
2009	-	-	471	-	-	-	-	-
2010	∞	1-∞	1244	-	∞	-	∞	-
2011	104	40-∞	-	-	113	-	121	-
2012	-	-	-	-	-	-	-	-
2013	-	-	335	-	-	-	-	-
Mean	72	-	827	0.045	76	0.048	96	0.060

Table 2.3 Comparison of N_e/N_c ratios for seal. N_b and N_e was estimated with contemporary, historical, and demographic methods. Our overall estimate of 0.048 was similar to other N_e/N_c ratios that used one sample contemporary estimators.

Species	N_e or N_b	N_e estimation method	N_c	N_e/N_c	Reference
<u>Contemporary</u>					
<i>Pusa hispida saimensis</i>	14.7	LDNE	310	0.05	Valtonen <i>et al.</i> 2014
<i>Pusa hispida saimensis</i>	113.0	Temporal method	310	0.36	Valtonen <i>et al.</i> 2014
<i>Platanthera hookeri</i>	842.9	ONESAMP	71,590	0.07	Collins <i>et al.</i> 2016
<i>Platanthera hookeri</i>	452.8	LDNE	71,590	0.04	Collins <i>et al.</i> 2016
<i>Phoca vitulina</i>	90.5	LDNE	995	0.09	Andersen <i>et al.</i> 2011
<u>Historical</u>					
<i>Arctocephalus australis</i>	13,613	Estimated with H_e	250,000–300,000	0.05	Robertson 2015
<i>Arctocephalus forsteri</i>	22,379	Estimated with H_e	200,000	0.11	Robertson 2015
<i>Arctocephalus gazella</i>	30,000	Estimated with H_e	≤6,200,000	<0.01	Robertson 2015
<i>Arctocephalus tropicalis</i>	37,330	Estimated with H_e	>310,000	0.12	Robertson 2015
<i>Callorhinus ursinus</i>	30,000	Estimated with H_e	<1,100,000	0.03	Robertson 2015
<i>Eumetopias jubatus</i>	9,563	Estimated with H_e	143,000	0.07	Robertson 2015
<i>Lobodon carcinophaga</i>	880,200	IMA2	15,000,000	0.06	Curtis <i>et al.</i> 2011
<i>Leptonychotes weddellii</i>	151,200	IMA2	800,000	0.19	Curtis <i>et al.</i> 2011
<i>Neophoca cinerea</i>	4,657	Estimated with H_e	13,790	0.34	Robertson 2015
<i>Ommatophoca rossii</i>	254,500	IMA2	220,000	1.16	Curtis <i>et al.</i> 2011
<i>Platanthera hookeri</i>	7,875	IMA2	71,590	0.11	Collins <i>et al.</i> 2016
<i>Platanthera hookeri</i>	9,563	Estimated with H_e	11,855	0.81	Robertson 2015
<i>Platanthera hookeri</i>	14,694	Estimated with H_e	11,855	1.23	Robertson 2015
<i>Platanthera hookeri</i>	7,490	DIYABC	11,855	0.63	Osborne <i>et al.</i> 2016
<i>Zalophus californianus</i>	6,968	Estimated with H_e	355,000	0.02	Robertson 2015
<i>Zalophus wollebaeki</i>	15,897	Estimated with H_e	20,000–40,000	0.54	Robertson 2015
<u>Demographic</u>					
<i>L. weddellii</i>	8.5- 10.0	Sex ratio equation	26	0.33-0.39	Gelatt <i>et al.</i> 2010
<i>A. australis</i>	2,153	Sex ratio equation	27,219 (1992)	0.08	de Oliveira <i>et al.</i> 2006
<i>A. australis</i>	2,153	Sex ratio equation	24,481 (1996)	0.09	de Oliveira <i>et al.</i> 2006

*Estimated with H_e (Lehmann *et al.* 1998), ONESAMP (Tallmon *et al.* 2008), IMA2 (Hey 2010), LDNE (Waples & Do 2008), temporal method (Jorde & Ryman 2007), DIYABC (Cornuet *et al.* 2010), sex ratio equation (Wright 1931, 1938)

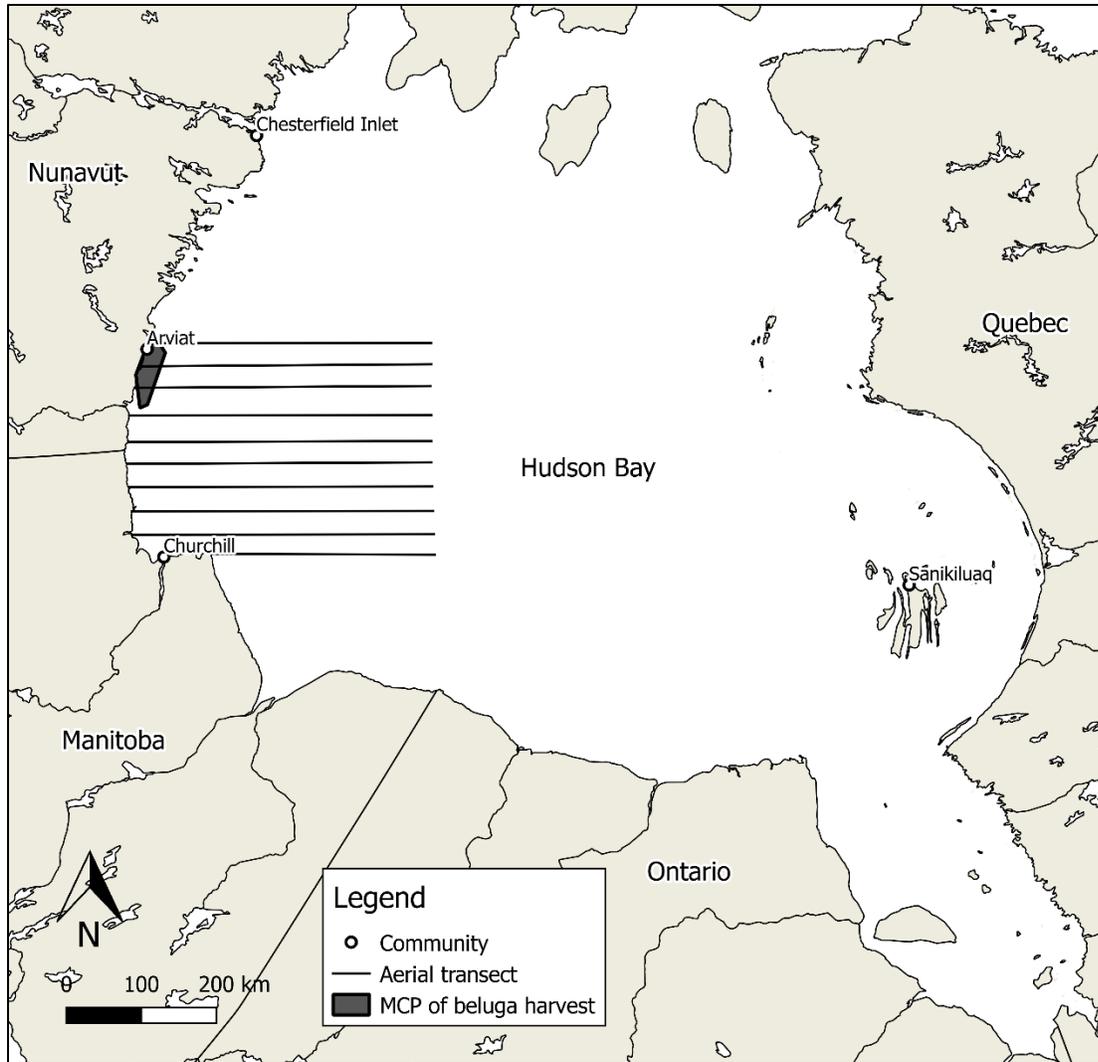


Figure 2.1 Ringed seal (*Pusa hispida*) tissue samples were collected from the area surrounding Arviat, Nunavut. Tissue samples were collected through Fisheries and Oceans Canada’s community-based monitoring program. 509 samples were used to estimate N_b in Hudson Bay. A minimum convex polygon (MCP) was calculated for all points where beluga (*Delphinapterus leucas*) were harvested to approximate the area ringed seals were collected from. Aerial transects were flown between Churchill, Manitoba and Arviat, Nunavut to estimate density and abundance.

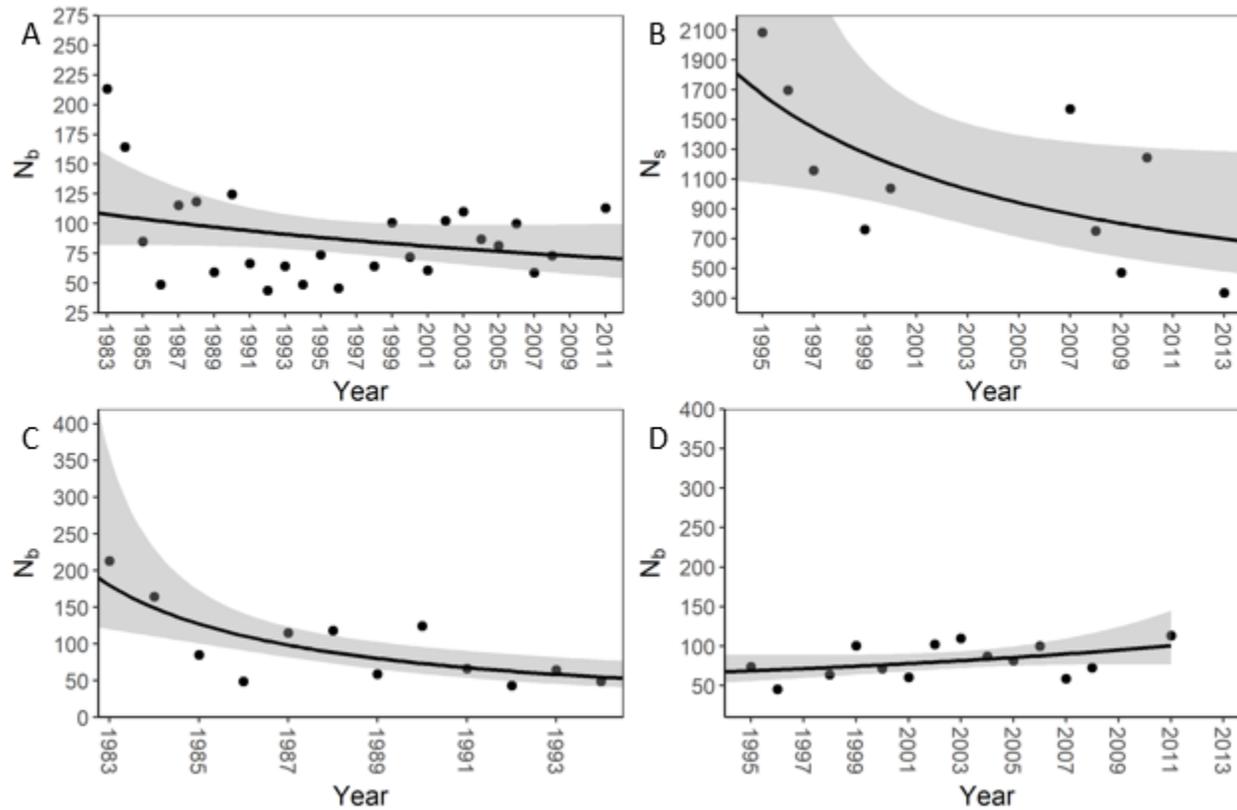


Figure 2.2 Changes in the effective number of breeders (N_b) and the survey population size (N_s) over time. A) The relationship between N_b and time indicated that N_b is stable in northwestern Hudson Bay ($R^2 = 0.077$, $T_{25} = -1.27$, $p = 0.218$). B) Relationship between N_s and year ($R^2 = 0.368$, $T_8 = -2.12$, $p = 0.067$). C) Relationship between N_b and year, before aerial surveys were conducted ($R^2 = 0.539$, $T_{10} = -3.52$, $p = 0.006$). D) Relationship between N_b and year, at the time aerial surveys were flown ($R^2 = 0.184$, $T_{13} = 1.70$, $p = 0.115$).

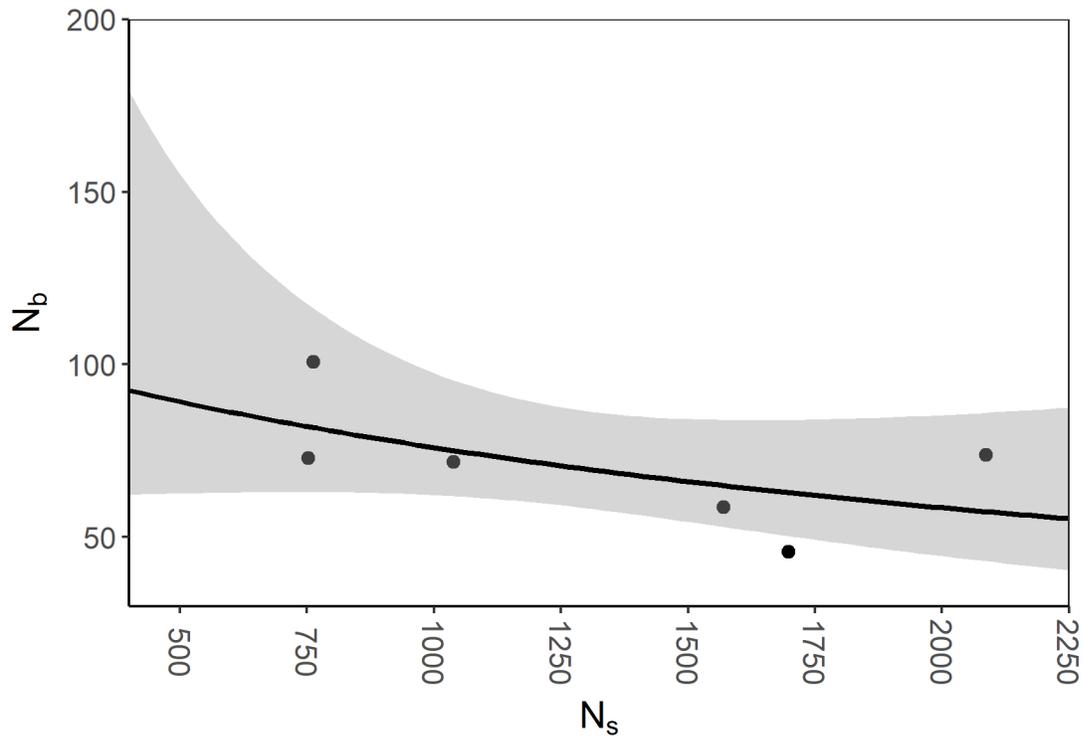


Figure 2.3 The relationship between the effective number of breeders (N_b) and the survey population size (N_s) of ringed seals in northwestern Hudson Bay. Contrary to our expectations, N_b did not increase at larger values of N_s ($R^2 = 0.543$, $F_5 = 2.04$, $p = 0.097$).

Chapter 3: Precipitation influences the effective number of breeders in ringed seals in western Hudson Bay, Canada

Abstract

Environmental variables have a profound influence on the survival and reproductive success of Arctic organisms. Weather variables can affect juvenile ringed seal survival, which will determine the number of breeders that contribute to the population as a surviving mature cohort. Here we used the effective number of breeders (N_b), estimated with sibship assignment, to examine the effect that environmental variables have on recruitment of ringed seals to breeding age. N_b reflects the number of individuals contributing genetically during one breeding season in ringed seals. Our results indicated that increased spring rainfall decreased N_b , while greater snow depth increased N_b . There was a lag between rain and N_b of 5 years and 6 years between snow depth and N_b . There was a positive relationship between the date of fall freezeup and N_b , indicating that later freezeup increased N_b with a lag of 7 years. Date of fall freezeup predicted N_b better than the date of spring breakup. Our results suggest that the projected decreased snow cover and increased spring rainfall may increase mortality of pups during their first months of life. Snow cover may provide protection from predators, but spring rainfall collapses lairs, which can kill pups. However, the later date of fall freezeup may increase survival of ringed seals through their first winter. Later fall freezeup delays when polar bears can start hunting ringed seals from the sea ice and may lead to higher primary productivity and food availability. As climate change becomes more pronounced in the Arctic, an increase in spring rainfall and decrease in snow depth may have a negative effect on ringed seal recruitment.

Introduction

Population dynamics of Arctic species are influenced by climate and annual weather patterns (Stearns 1992; Post *et al.* 2009). Ringed seals (*Pusa hispida*) have adapted to the extreme environmental conditions of the Arctic. However, the current trend toward a warmer Arctic raises concern that ringed seals will struggle to adapt to the environmental changes associated with climate change (Stirling & Smith 2004; Ferguson *et al.* 2005; Kovacs & Lydersen 2008). Recent aerial survey estimates in northwestern Hudson Bay have suggested that ringed seal populations may be declining (Chambellant *et al.* 2012a; Young *et al.* 2015). However, ringed seals are a difficult species to survey visually because they have cryptic behaviour, spending a lot of time underneath ice and water. Unobserved seals represent an unknown proportion of aerial surveys, in addition to other sources of bias caused by the meteorological conditions, observer proficiency, the time of day surveys are flown, whether or not the survey occurred during peak haul-out, and noise from aircraft flown (Smith & Hammill 1981; Frost *et al.* 2004; Krafft *et al.* 2007; Chambellant *et al.* 2012a). Additionally, as spring breakup continues to occur earlier, the detectability of ringed seals may be reduced if more seals are in the water during aerial surveys – negatively biasing density estimates.

During the ice-covered season, female ringed seals construct lairs under the snow that accumulates on sea ice, to provide shelter while they breed, raise pups, and rest (Kovacs & Lydersen 2008). Lairs are most commonly constructed along pressure ridges where more snow accumulates (Smith & Stirling 1975). The lairs provide protection from predators and the harsh climate and are integral to pup survival (Smith 1975; Smith *et al.* 1991). Mature females give birth to one pup annually, between mid-March and mid-April (Smith & Stirling 1975; Vincent-

Chambellant 2010). Ringed seals wean their pups six weeks after birth (Hammill *et al.* 1991), followed by mating in May (Lydersen 1995), moulting in June (Smith 1973), and delayed implantation occurs in August or September (Smith 1987). In summer, during the open water season, adult seals feed intensively to replenish their fat stores, before establishing territories in the autumn that they maintain during the winter (Smith 1987; Young and Ferguson 2013).

Several studies have found that the timing of spring breakup was linked to recruitment and body condition in ringed seals. The proportion of young-of-the-year in the fall harvest decreased when breakup occurred earlier in western Hudson Bay (Ferguson *et al.* 2005, 2016) and in the east Amundsen Gulf and west Prince Albert Sound (Harwood *et al.* 2012) and the Alaskan Bering and Chukchi seas (Crawford *et al.* 2015). Conversely, earlier spring breakup was associated with higher body condition and increased ovulation rates in the eastern Beaufort Sea (Harwood *et al.* 2000, 2012) and may lead to higher recruitment, body condition, and length in the Chukchi and Bering seas (Stirling *et al.* 1977; Kingsley *et al.* 1985; Stirling 2002; Crawford *et al.* 2015). Additionally, cortisol levels in whiskers of harvested seals have increased in western Hudson Bay as spring breakup has occurred earlier (Chambellant *et al.* 2012; Ferguson *et al.* 2017). The difference in responses between regions may be explained by a curvilinear pattern that suggests that there is an optimal length of the ice-free season for ringed seals (Chambellant *et al.* 2012b). As Hudson Bay is in the southern extent of ringed seals' range, lower ice concentration is expected to drive negative demographic and physiological responses (Ferguson *et al.* 2017). The date of fall freezeup may also affect ringed seal survival, although the effect of this variable on ringed seal populations has been explored less than the date of spring breakup. Stirling & Øristland (1995) suggested that polar bears in Hudson Bay may kill ringed seals at

higher rates immediately before and after their 4-month fast. However, due to limited daylight during fall, quantification of kill rates is challenging. Ringed seal body condition index declined in 1992, when there was a very short open water season in Hudson Bay (Chambellant *et al.* 2012b).

Precipitation and warmer spring temperatures have been linked to lower recruitment in western Hudson Bay (Ferguson *et al.* 2005, 2016; Iacozza & Ferguson 2014). Similar to the date of spring breakup, an optimal snow depth for pup survival has been proposed for ringed seals (Chambellant *et al.* 2012b). A thin snow layer over dens results in increased predation by polar bears (Ferguson *et al.* 2005). A thick snow layer over dens could reduce air circulation and cause pups to swim to another den (Kelly & Quakenbush 1990). Before pups have developed their subcutaneous blubber, it is energetically expensive for them to return to a state of homeostasis after swimming between lairs (Kelly & Quakenbush 1990). As temperatures become warmer in spring more precipitation will be falling as rain. Rain has been shown to increase polar bear hunting success on ringed seals and caused dens to collapse (Hammill & Smith 1991; Stirling & Smith 2004). Collapsed dens can expose ringed seal pups to harsh environmental conditions (Kovacs & Lydersen 2008) and predation by avian predators (Lydersen & Smith 1989).

Chambellant *et al.* (2012b) and Crawford *et al.* (2015) found that age at maturity has declined from 5.4 to 3.8 years and 6.0 to 3.9 years, respectively – as the length of the ice-free season has increased. The decreased age at maturity and juvenile mortality are indicative of a population in a growth phase after a period of lower density (Oli & Dobson 1999; Chambellant *et al.* 2012b). Less intraspecific competition at lower densities increases the resources available per individual and allows them to reach maturity at younger ages (Trippel 1995). Additionally, in

warmer years when sea-ice thickness is less and the duration of ice cover is shorter, primary productivity may be higher (Arrigo *et al.* 2008; Pabi *et al.* 2008) and polar bear predation reduced, which may lead to increased growth rates and survival (Stirling & Archibald 1977). Recent aerial survey estimates in northwestern Hudson Bay have suggested that ringed seal populations may be declining (Chambellant *et al.* 2012a; Young *et al.* 2015).

Genetic methods of monitoring and assessing population dynamics have improved rapidly over the last several decades (Schwartz *et al.* 2006). They enable us to investigate demographic and evolutionary processes that are otherwise logistically impractical to study (Schwartz *et al.* 2006). Genetic monitoring and assessment methods can be comparatively inexpensive and allow us to gain a large amount of information from relatively few samples (Waples & Do 2008; Palstra & Fraser 2012). One of the most promising parameters for population monitoring is N_e (Schwartz *et al.* 2006; Yates *et al.* 2017). N_e is the size of an ideal population that loses variation through genetic drift at the same rate as the population under consideration (Wright 1931) or roughly the number of individuals that contribute genetically to a given generation. N_e has been used to monitor the total number of individuals in a population or the census population size (N_c) (Boulanger *et al.* 2004; Perrier *et al.* 2016). Although variables aside from N_c can influence N_e (Palstra & Fraser 2012), population size is a strong driver of N_e (Frankham 1995; Kalinowski & Waples 2002). While genetic methods of monitoring have limitations (Wang 2016; Waples 2016b), they are not subject to the biases introduced by environmental conditions as surveys, which rely on counts of individuals. Furthermore, even if the mean estimates and upper confidence interval estimates of N_e are infinite, estimating effective population size can still be useful in conservation as the bottom interval is more stable

and can be a useful indicator of trends (Waples & Do 2010). However, N_e is notoriously difficult to estimate, so N_b , which is easier to estimate, is frequently used (Waples & Antao 2014). N_b is the effective number of breeders per reproductive event (Waples 1990). N_b is similar to N_e , but applies to one reproductive event, whereas N_e applies to a generation (Waples 2005). Therefore, N_b is more useful when investigating the effects of annual weather variation when samples can be aged and divided into birth years. While samples collected through subsistence harvest are representative of the population (Smith 1973; Young & Ferguson 2014), N_b quantitatively represents the number of individuals that successfully breed in a season.

Although several studies have investigated the relationship between recruitment and environmental variables using the proportion of young-of-the-year seals in the fall harvest (Stirling *et al.* 1999; Ferguson *et al.* 2005; Crawford *et al.* 2015), none have investigated these relationships using a genetic monitoring approach. Genetic monitoring may be advantageous over aerial surveys because the results are not subject to biases introduced by weather conditions at the time surveys are flown. Our objective is to examine the effects that environmental variables have on recruitment of ringed seals in western Hudson Bay, using as N_b . We use a time series of N_b estimates to explore the relationship between N_b and date of spring breakup, date of fall freezeup, yearly average snow depth, and spring rainfall. These variables have been shown to influence ringed seal recruitment in studies that use the proportion of young-of-the-year in the subsistence harvest (Stirling & Smith 2004; Ferguson *et al.* 2005, 2016; Iacozza & Ferguson 2014). Since mortality of ringed seals is the highest during the first year after birth (Pilfold *et al.* 2012), we assumed that environmental variables would most strongly affect mortality of young-of-the-year seals, as has been shown with polar bears (Regehr *et al.* 2007). If recruitment is

linked to changing environmental factors such as timing of spring breakup, freezeup, snow depth, or spring rainfall, then N_b should respond to the trends of these predictive variables. Investigating the impact of annual variation of environmental variables on ringed seals from a genetic perspective may offer insight into how ringed seals will respond to climate change.

Methods

Sample collection and study area

Samples were collected through Fisheries and Oceans Canada's community-based monitoring program. We obtained tissue samples from subsistence hunters that they collected during the fall harvest. Sample collection in fall is most representative of the demographic structure of the population as hunters tend not harvest selectively and seals of all age groups congregate together (Smith 1973). Hunters also collected the lower jaws, so seals could be aged. The samples used were collected from Arviat, Nunavut (61.1078° N, 94.0624° W) (Figure 3.1).

Seals were aged by Matson's laboratory in Montana, USA using the cementum method (Stirling *et al.* 1977; Bernt *et al.* 1996; Stewart *et al.* 1996). The right canine was used for aging, unless it was unavailable. When the right canine was unavailable the preference of tooth to use followed the order: left canine, right or left 1st postcanine tooth, or a postcanine double-rooted tooth (Chambellant & Ferguson 2009). Ages and collection years were used to determine the birth year of each seal, as estimation of N_b requires a sample of individuals born in the same reproductive event (Jones & Wang 2010) (Table 3.1).

DNA extraction and amplification

Genomic DNA was extracted using a Thermo Scientific GeneJET Genomic Purification Kit® (Thermo Scientific, Nashville, USA). Polymerase chain reaction (PCR) conditions were

optimized for 14 polymorphic microsatellite loci (Appendix A: Table A2.). PCR runs consisted of an initial denaturing step at 95 °C for 5 minutes, followed by 32 cycles with a 5-minute 95 °C denaturing step, a 1-minute annealing step at the temperatures listed in Table A2 (Appendix A), and a 45-second DNA synthesis step at 72 °C. These cycles were followed by a 30-minute extension step at 72 °C. All PCR runs were performed on a C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario, Canada). PCR products were then visualized using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). To prepare samples for analyzer runs, we added 1 *ul* of PCR product to a mixture consisting of 40 *ul* of GeneScan™ 600 LIZ® dye Size Standard (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and 1000 *ul* of Hidi™ Formamide (Applied Biosystems). After samples were run on the analyzer all genotypes were scored using GeneMarker (SoftGenetics, State College, PA).

Microsatellite analysis

To test loci for the presence of homozygote excess, null alleles, large allele dropout, and stutter we used MICRO-CHECKER (Van Oosterhout *et al.* 2004). GENEPOP v 4.2.2 (Raymond & Rousset 1995; Rousset 2008) was used to test for deviations from Hardy-Weinberg equilibrium (HWE). To account for multiple pairwise comparisons in HWE, we adjusted α using Holm's sequential Bonferroni correction (Holm 1979; Rice 1989) with an α of 0.05 .

We used the program COLONY2 (Jones & Wang 2010), which implements the sibship assignment method (Wang 2004, 2009), to estimate N_b . We used COLONY2 to estimate N_b , as it is the most accurate estimator when sample sizes are limited (Wang 2016). We used PEDANT (Johnson & Haydon 2007) to estimate the rate of null alleles and long allele dropout, which are required to run COLONY2. The analysis method used was full-likelihood, non-random mating

was assumed, and inbreeding was not selected (Jones & Wang 2010), as it has not been detected in Hudson Bay ringed seals (Petersen 2008). Ringed seals are likely slightly polygynous (Kelly *et al.* 2010), with females typically giving birth to one offspring in a year, while males can sire several (Krafft *et al.* 2007). Therefore, we selected female monogamy and male polygyny when running COLONY2.

Environmental data

We obtained spring ice breakup and fall freezeup dates from Young *et al.* (2015). Following previous protocols (Etkin 1991; Stirling *et al.* 1999; Young *et al.* 2015), breakup was defined as the date that annual sea-ice concentration decreased to and remained below 50% in the study area and vice-versa for freezeup. Breakup and freezeup data were available from 1975 to 2015 for northwestern Hudson Bay, as defined by Canadian Ice Services (Figure 3.1). We extracted Arviat daily rainfall data (Environment and Climate Change Canada 2018) for April and May and calculated the average rainfall per day. Rainfall data was available from 1985 to 2015, excluding 2009, 2012, and 2014. We obtained snow-depth estimates from Iacozza & Ferguson (2014), which were acquired from the Advanced Microwave Scanning Radiometer – Earth (AMSR-E) at nsidc.org/data/docs/daac/nsidc0447_CMC_snow_depth/index.html (Brown & Brasnett 2015). We used the average snow depth for the entire winter in years where AMSR-E data was available (2003-2010).

Statistical analysis

All data analysis was conducted in RSTUDIO 1.1.383 (RStudio Team) with R version 3.3.1 (R Core Team 2016). Prior to analysis, all predictor variables were screened for multicollinearity using the function *chart.Correlation* from PerformanceAnalytics (Peterson &

Carl 2014), and *afc* from base R was used to check for autocorrelation of the residuals from all models. We used the function *glm()* from base R to fit generalized linear models. As relationships between several variables were non-linear and all variables were continuous we used the Gamma distribution with an inverse link function. We standardized all predictor variables to a mean of 5 and a standard deviation of 1, prior to fitting models. Variables were centered around a mean of 5 to ensure that all values were positive as is required to run generalized linear models with Gamma distributions. N_b was treated as the response variable and snow depth, date of spring breakup, date of fall freezeup, and spring rainfall as explanatory variables. Because of the limited number of observations available for snow depth, model selection was conducted with separately from the amount of spring rainfall, and the dates of fall freezeup, and spring breakup. To account for the variability in age at maturation, we tested for relationships between N_b and environmental variables with lags in N_b of 2 to 7 years, as female ringed seals reach maturity at 2 to 4 years and males 5 to 7 years (Gjertz & Lydersen 1986; Smith 1987; Holst *et al.* 1999; Krafft *et al.* 2006b; Chambellant *et al.* 2012b). The function *AICctab* from the package *bbmle* (Bolker & R Development Core Team 2010) was used to compare the relative strength of models with Akaike information criterion corrected for small sample sizes (AICc). Cox-Snell pseudo R^2 values (Snell & Cox 1989) were generated for top models with *RsqGLM* from *modEvA* (Barbosa 2015). We pooled models from all lags to examine the relative strength of relationships of N_b to environmental variables and to determine which lags were supported by the data. Models with an $\Delta AICc < 2$ were considered competing models (Anderson & Burnham 2002).

Results

MICRO-CHECKER detected that scoring errors due to stutter may have been present at locus Hg6.1, which we dropped from further analyses. Estimates of N_b with finite means ranged from a minimum of 44 in 1992 to a maximum of 213 in 2010 (Table 3.1). We retained all mean estimates of N_b that did not include infinity, as sensitivity analysis with COLONY2 has indicated that even when $n \leq 20$, robust estimates of N_b can still be obtained (Ozerov *et al.* 2015). We did not detect autocorrelation, correlations between environmental variables were low (Appendix B: Figure B1), and variance inflation factors were below 10 (Zuur *et al.* 2010). Therefore, we retained all explanatory variables. From 1974 to 2015, the date of spring breakup has occurred earlier ($R^2 = 0.219$, $T_{39} = 3.41$, $p = 0.002$) and fall freezeup has occurred later ($R^2 = 0.232$, $T_{39} = -3.35$, $p = 0.002$) (Figure 3.2). No significant temporal trends occurred in either spring rainfall or snow depth (rainfall: $R^2 = 0.023$, $T_{26} = 0.46$, $p = 0.649$, snow depth: $R^2 = 0.191$, $T_6 = 1.14$, $p = 0.298$) (Figure 3.2).

When models with snow depth were excluded, spring rain (with a 5-year lag) and the date of fall freezeup (with a 7-year lag) were the most important predictors of N_b (Tables 3.2, 3.3). At a lag of 5 years, the amount of spring rainfall negatively affected N_b ($R^2 = 0.359$, $T_9 = 2.25$, $p = 0.051$), and the date of fall freeze with a 7-year lag positively affected N_b ($R^2 = 0.593$, $T_9 = -3.68$, $p = 0.006$) (Figure 3.3). When snow depth was analyzed separately, a 6-year lag of N_b relative to snow depth was the most likely model explaining variation in N_b (Table 3.4), and snow depth was a significant predictor of N_b ($R^2 = 0.635$, $T_5 = 3.00$, $p = 0.030$) (Table 3.5, Figure 3.3). No models including the dates of sea ice breakup received an AICc score < 2 .

Discussion

Our results indicate that in western Hudson Bay, when lags are considered, increased spring rainfall negatively affects ringed seal N_b and that snow depth and the date of fall freezeup have positive relationships with N_b . Spring rainfall has been noted to affect survival by collapsing lairs during spring, which increases exposure of young seals to predators (Lydersen & Smith 1989; Stirling & Smith 2004) and harsh conditions (Kelly 2001). The link between snow greater snow depth and higher recruitment has been established previously (Furgal *et al.* 1996; Ferguson *et al.* 2005; Iacozza & Ferguson 2014). We observed a lag between environmental variables and the response of N_b and suggest this is due to the time it takes ringed seals to reach maturity and start contributing to N_b by breeding. Since ringed seal mortality is highest when they are young, environmental variables can have a disproportionate effect on the survival of a given cohort. If environmental conditions are favourable and juvenile mortality is low, N_b will increase once a given cohort reaches maturity and starts breeding – or vice-versa if conditions reduce juvenile survival.

Our results indicated that the amount of spring rainfall is one of the primary mechanisms influencing ringed seal recruitment and pup/juvenile survival, unlike previous studies that have examined the relationship between recruitment and environmental variables in ringed seals (Stirling *et al.* 1999; Ferguson *et al.* 2005; Stirling 2005). Spring rainfall potentially impacts seals through top-down effects, by making them more vulnerable to predators in their subnivean lairs (Lydersen & Smith 1989; Hammill & Smith 1991; Stirling & Smith 2004). While hunting success of polar bears on ringed seals appears to be higher when rainfall increases (Stirling & Smith 2004), hunting success of Arctic foxes (*Vulpes lagopus*) (Smith & Lydersen 1991) and

avian predators also increases (Lydersen & Smith 1989). Rain can also increase mortality of young seals when lairs collapse (Stirling & Smith 2004). The increase in mortality that these biotic and abiotic interactions cause likely limits the number of ringed seals in the future breeding cohort, reducing N_b .

Spring rainfall may also influence ringed seal population dynamics through bottom-up effects by influencing prey communities. Ringed seal diet in western Hudson Bay varies seasonally, with benthic prey being more important in winter then transitioning to pelagic prey during the open water season (Young & Ferguson 2013). Although ringed seals in western Hudson Bay feed primarily on sand lance (*Ammodytes* spp.), capelin (*Mallotus villosus*) and Arctic cod (*Boreogadus saida*) are also important prey species in the spring (Chambellant *et al.* 2013). Young ringed seals feed on zooplankton more than adults, who consume more forage fish (Yurkowski *et al.* 2016a). Baird (1990) found that sea birds that forage on similar prey to ringed seals had reduced recruitment in years with more spring and summer rainfall. If rainfall has a detrimental effect on prey abundance or causes them to be less accessible, then young ringed seals that rely on them in spring may be negatively affected. Much of the expected increase in Arctic precipitation over the 21st century is predicted to fall as rain (Hezel *et al.* 2012). It should be noted that our study area was in the southern extent of ringed seals distribution and that rain may be less of a concern in more northern regions.

Our finding that increased snow depth leads to higher N_b in future years may suggest that the foraging efficiency of predators on seals in subnivean lairs is reduced when snow depth is greater (Furgal *et al.* 1996; Ferguson *et al.* 2005). Lairs with less snow on them provide less protection from the environment and have a higher probability of detection through scent or

sound by polar bears. Additionally, ringed seal pup survival has been shown to be greater in years with a longer duration of snow cover (Iacozza & Ferguson 2014). However, there may be an optimal snow depth for ringed seal recruitment (Ferguson *et al.* 2005; Chambellant *et al.* 2012b). Under future climate projection scenarios, cumulative snowfall is not expected to decline overall, but snow depth on sea ice is projected to in April (Hezel *et al.* 2012; Bintanja & Selten 2014).

Ringed seals feed intensively during summer and fall to maximize their fat stores before the less productive winter months (Ryg *et al.* 1990; Young & Ferguson 2013). The positive relationship between the later date of fall freezeup and N_b may reflect an increase in the length of hunting season for ringed seals and thus a longer foraging period to increase fat stores before winter, leading to greater survival of young animals. Diet varies among age classes, with young ringed seals feeding on zooplankton more than adults, who consume primarily forage fish (Yurkowski *et al.* 2016a). Since later fall freezeup leads to higher production of ice algae, phytoplankton, zooplankton, and benthic species (Tremblay *et al.* 2011), young ringed seals may benefit from the increased availability of zooplankton and other prey items that occur in years with a later fall freezeup. They may also benefit from the decreased time that polar bears, which rely on sea ice as a platform for hunting seals (Stirling & Øristland 1995), can spend hunting ringed seals in fall.

Although the effect that environmental variables have on ringed seal recruitment and density have been investigated (Ferguson *et al.* 2005; Chambellant *et al.* 2012a; Iacozza & Ferguson 2014; Young *et al.* 2015), the effect that they have on N_b has not. Using N_b allowed us to investigate the relationship between ringed seal population dynamics and environmental

variables, without our results being confounded by the same environmental conditions that can bias estimates from aerial surveys (Born *et al.* 1999; Krafft *et al.* 2006a; Chambellant *et al.* 2012a; Young *et al.* 2015). Our study provides evidence that snow depth, spring rainfall, and the date of fall freezeup are related to recruitment to breeding age, but that lag effects should be considered when exploring demographic responses of ringed seals to environmental variables. The lag between environmental variables and N_b is likely because weather conditions have a large impact on pup and juvenile survival, which affects the recruitment of individuals from that cohort to breeding age. Delayed effects of environmental variables on population recruitment and future reproductive rates have also been documented extensively in piscivorous Arctic seabirds that have delayed age at maturity (Sandvik *et al.* 2012; Erikstad *et al.* 2013; Zador *et al.* 2013).

N_b is difficult to estimate in wild populations, as the simplifying assumptions that estimation methods rely on are typically violated (Wang 2016). Populations with a large number of individuals are particularly problematic, as the genetic drift signal is often weak and requires a substantial, often unfeasibly large sample size to reduce noise in estimates (Waples 2016b). Another difficulty in estimating N_b is determining which spatial scale an estimate applies to. N_b generally apply to a local scale or to the spatial scale of the parents that a sample was taken from (Waples & England 2011). We did not have telemetry tracking data for the seals that we collected samples from, but Yurkowski *et al.* (2016) determined that ringed seals in Hudson Bay (sampled from Sanikiluaq) primarily remained in a residency state during the open water season and Kelly *et al.* (2010) demonstrated that adult ringed seals have some degree of breeding-site

fidelity. Therefore, we assumed that ringed seals sampled in our study were local and did not undertake large migrations.

The expected changes in environmental conditions and the subsequent changes in ringed seal population dynamics will present a challenge for resource users and managers in coming years. However, without methods for monitoring ringed seal population size, declines in population size will not be detected. Our study addresses this knowledge gap by demonstrating the responses of N_b to the amount of spring rainfall, snow depth, and the dates of fall freezeup and spring breakup. However, given the limitations of current N_b estimators (Gilbert & Whitlock 2015; Wang 2016; Waples 2016), further research exploring the relationship between environmental variables and ringed seal population dynamics is needed to determine how climate change will affect them.

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Table 3.1 Effective number of breeders (N_b) for each yearly cohort was estimated with 95% confidence intervals for a sample size (n). N_b was estimated using COLONY2 with long runs of the full-likelihood method, assuming non-random mating, polygyny, and no sibship or N_e priors.

Year	N_b	Lower CI	Upper CI	n
1981	∞	1	∞	5
1982	∞	1	∞	5
1983	∞	1	∞	6
1984	213	67	∞	10
1985	164	53	∞	9
1986	85	27	∞	7
1987	49	25	121	18
1988	115	55	504	18
1989	119	57	623	19
1990	59	28	305	14
1991	125	33	∞	7
1992	67	33	205	17
1993	44	20	185	15
1994	64	30	914	15
1995	49	21	405	11
1996	74	24	∞	6
1997	46	20	798	12
1998	∞	1	∞	7
1999	64	27	∞	10
2000	101	41	∞	12
2001	72	42	112	32
2002	61	35	91	33
2003	102	62	150	45
2004	110	63	193	32
2005	87	52	129	40
2006	82	34	602	16
2007	100	41	2630	13
2008	59	31	99	25
2009	73	37	216	18
2010	-	69	∞	12
2011	∞	1	∞	3
2012	113	40	∞	11

Table 3.2 Comparison of generalized linear models to predict N_b . Predictive variables included: the date of spring breakup (Break), the date of fall freezeup (Freeze), and the average mm of rainfall that occurred in April and May annually (Rain). Models were compared to determine which models fit the data best with lags 2-7. Sample size (n), number of parameters (K), Akaike's information criterion corrected for small sample sizes (AICc), delta AICc (Δ AICc), log-likelihood (L), and AIC weight (W_i).

Rank	Lag	Model parameters	n	K	AICc	Δ AICc	W_i	L
1	7	Freeze	11	3	97.5	0.0	0.405	-44.1
2	5	Rain	11	3	99.0	1.5	0.195	-44.8
3	5	Null	11	2	100.0	2.4	0.120	-47.2
4	6	Rain	11	3	102.2	4.7	0.039	-46.4
5	3	Null	11	2	103.0	5.4	0.027	-48.7
6	5	Break	11	3	103.1	5.6	0.025	-46.8
7	2	Null	11	2	103.1	5.6	0.025	-48.8
8	6	Null	11	2	103.5	5.9	0.021	-49.0
9	7	Null	11	2	103.5	6.0	0.021	-49.0
10	5	Freeze	11	3	103.8	6.3	0.018	-47.2
11	4	Null	11	2	103.9	6.4	0.017	-49.2
12	7	Break	11	3	104.1	6.6	0.015	-47.3
13	5	Break + Rain	11	4	104.2	6.7	0.014	-44.8
14	6	Break + Rain	11	4	105.8	8.3	0.007	-45.6
15	4	Rain	11	3	105.9	8.4	0.006	-48.2
16	3	Break	11	3	106.1	8.6	0.006	-48.3
17	2	Rain	11	3	106.5	8.9	0.005	-48.5
18	2	Freeze	11	3	106.6	9.1	0.004	-48.6
19	3	Rain	11	3	106.7	9.2	0.004	-48.6
20	2	Break	11	3	106.8	9.2	0.004	-48.7
21	3	Freeze	11	3	106.9	9.3	0.004	-48.7
22	7	Break + Rain	11	4	107.0	9.5	0.004	-46.2
23	6	Freeze	11	3	107.2	9.7	0.003	-48.9
24	4	Break	11	3	107.3	9.7	0.003	-48.9
25	6	Break	11	3	107.4	9.8	0.003	-49.0
26	7	Rain	11	3	107.4	9.9	0.003	-49.0
27	4	Freeze	11	3	107.6	10.0	0.003	-49.1
28	4	Break + Rain	11	4	111.1	13.5	<0.001	-48.2
29	3	Break + Rain	11	4	111.3	13.8	<0.001	-48.3
30	2	Break + Rain	11	4	111.7	14.2	<0.001	-48.5

Table 3.3 Results from the top 2 generalized linear models, showing the effect of average daily spring rainfall with a 5-year lag and the date of fall freezeup with a 7-year lag on N_b . Numbers in parenthesis represent standard error.

	Freezeup	Rain
Fixed-effects estimates (SE)		
Intercept	0.031 (0.005)***	-0.002 (0.007)*
Parameter	-0.003 (0.001)**	0.003 (0.001)*
R^2	0.593	0.359
n	11	11

SE = standard error, R^2 = coefficient of determination, n = number of observations, significance codes: <0.001***, 0.01**, 0.05 *.

Table 3.4 Comparison of generalized linear models to determine which lag between snow depth and N_b best predicted N_b ($n = 8$). Akaike's information criterion corrected for small sample sizes (AICc), delta AICc – the difference between AIC value and the smallest AIC in all models ($\Delta AICc$), log-likelihood (L), AIC weight (W_i), number of parameters (K).

Rank	Lag	Parameters	K	AICc	$\Delta AICc$	W_i	L
1	5	Null	2	67.0	0.0	0.362	-30.3
2	7	Null	2	67.3	0.3	0.313	-30.5
3	6	Snow	3	68.5	1.5	0.170	-28.3
4	6	Null	2	70.0	3.0	0.082	-31.8
5	5	Snow	3	72.4	5.4	0.024	-30.2
6	7	Snow	3	72.8	5.8	0.020	-30.4
7	3	Null	2	73.8	6.7	0.013	-33.7
8	4	Null	2	74.6	7.6	0.008	-34.1
9	2	Null	2	75.3	8.3	0.006	-34.5
10	3	Snow	3	78.2	11.1	0.001	-33.1
11	4	Snow	3	79.6	12.5	<0.001	-33.8
12	2	Snow	3	80.0	12.9	<0.001	-34.0

Table 3.5 Generalized linear modelling of N_b and snow depth with a 6-year lag. Numbers in parenthesis are standard error.

Snow depth	
Fixed-effects estimates (SE)	
Intercept	0.033 (0.007)**
Snow depth	-0.002 (0.001)*
R^2	0.635
n	7

SE = standard error, R^2 = coefficient of determination, n = number of observations, significance codes: <0.001***, 0.01**, 0.05*.

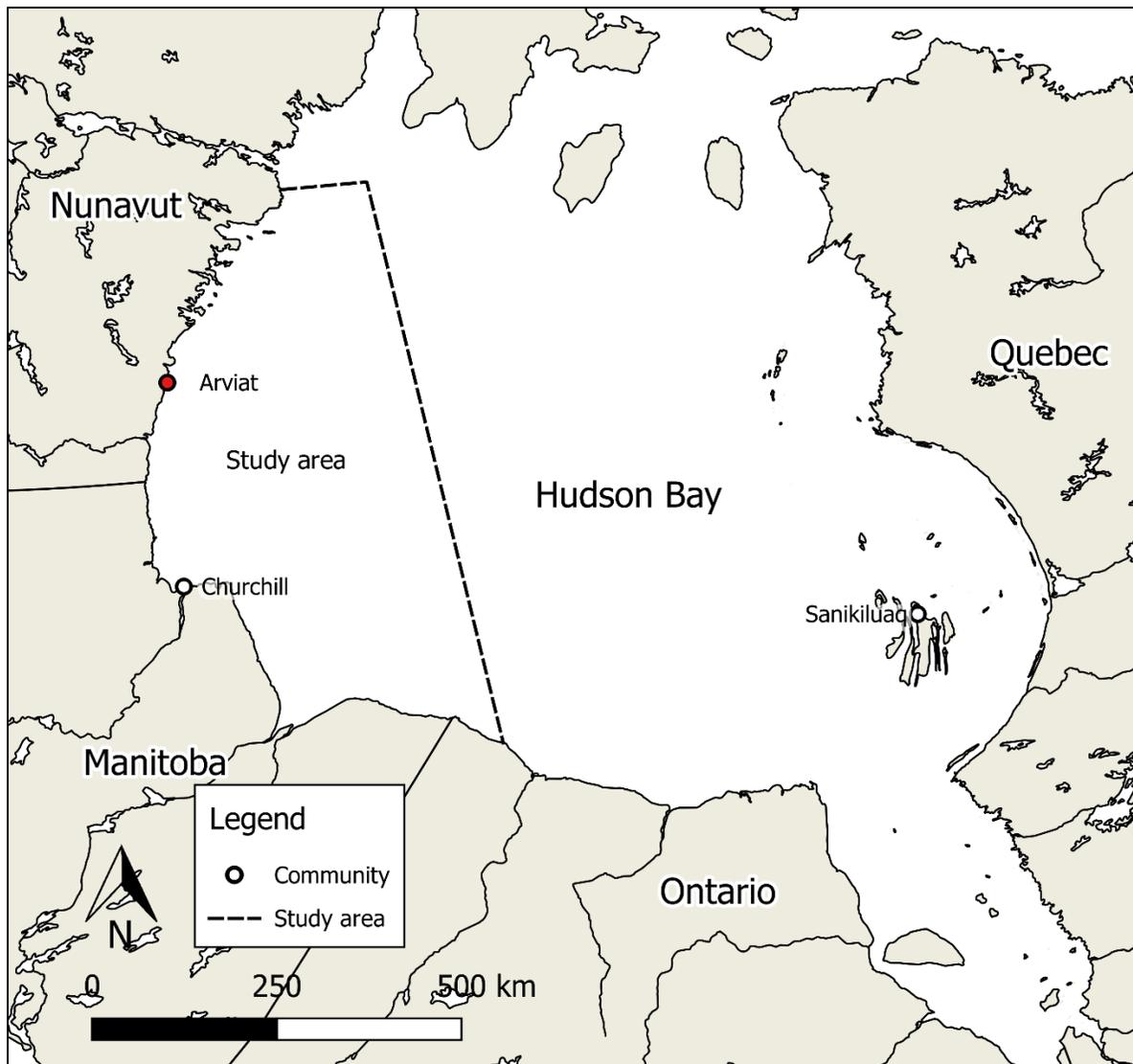


Figure 3.1 The samples used, and spring rainfall data were collected from Arviat, Nunavut (61.1078° N, 94.0624° W; red dot). The snow depth estimates and the dates of spring breakup/fall freezeup apply to northwestern Hudson Bay as defined by the study area (outlined by dashed line).

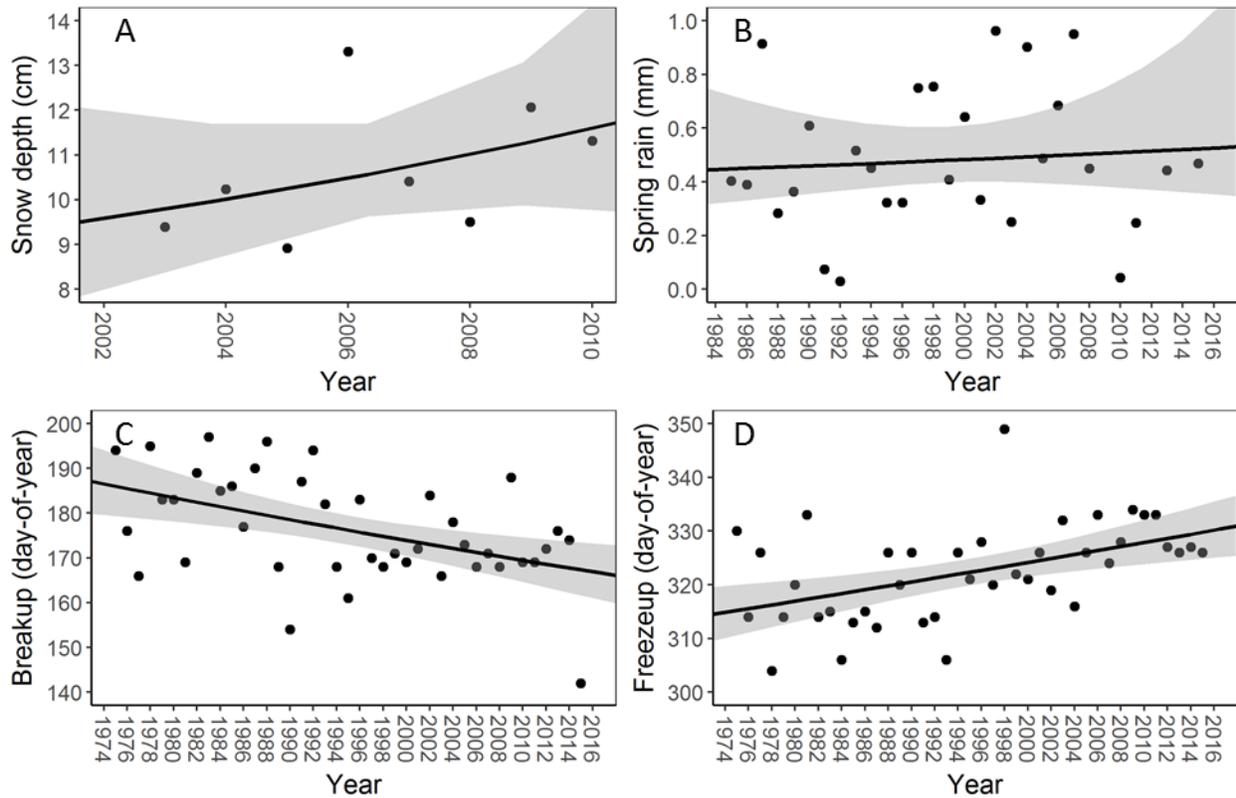


Figure 3.2 Changes over time in environmental variables from western Hudson Bay, Canada. No significant trend in A) snow depth ($R^2 = 0.191$, $T_6 = 1.14$, $p = 0.298$) or B) spring rainfall ($R^2 = 0.023$, $T_{26} = 0.46$, $p = 0.649$) occurred during our study. There were significant trends over time towards a longer open season, as C) spring breakup occurred earlier ($R^2 = 0.219$, $T_{39} = 3.41$, $p = 0.002$) and D) fall freezup later ($R^2 = 0.232$, $T_{39} = -3.35$, $p = 0.002$).

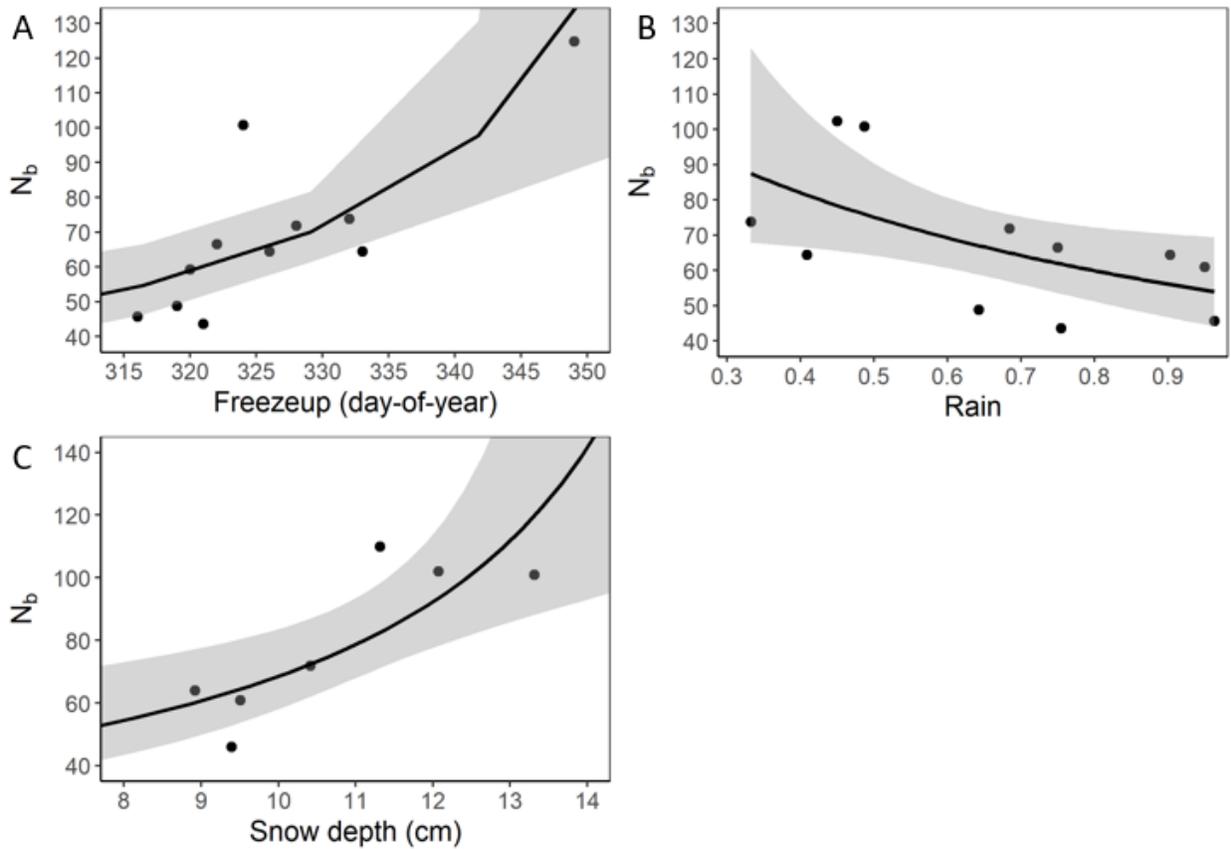


Figure 3.3 Relationship between number of breeders (N_b) and environmental variables were modelled using a Gamma distribution and an inverse link function. A) N_b increased with date of fall freezep with a 7- year lag ($R^2 = 0.593$, $T_9 = -3.68$, $p = 0.005$) and B) decreased with spring rainfall ($R^2 = 0.359$, $T_9 = 2.25$, $p = 0.051$) with a 5-year lag. C) The generalized linear model that received the highest AICc weight when snow depth was included, indicated that N_b was positively related to snow depth with a 6-year lag ($R^2 = 0.635$, $T_5 = 3.00$, $p = 0.030$).

Chapter 4: Thesis conclusion

In this thesis, I have implemented a genetic approach for monitoring population trends of ringed seals (*Pusa hispida*). While the method I used is more precise when applied to smaller populations, our overall result of the ratio between N_b and N_s was consistent with estimates from populations of Saimaa ringed seals (*Pusa hispida saimensis*), harbour seals (*Phoca vitulina*), and New Zealand sea lion (*Phocarctos hookeri*) (Andersen *et al.* 2011; Valtonen *et al.* 2014; Collins *et al.* 2016). However, we did not detect a positive relationship between our estimates of the effective number of breeders (N_b) and the number of seals counted in aerial surveys (N_s). We found that the amount of spring rainfall, snow depth, and the date of fall freezeup had an effect on N_b , while the date of spring breakup was not an important predictor. There were lags between environmental variables and N_b of 5, 6, and 7 years for spring rainfall, snow depth, and the date of fall freezeup respectively.

To estimate N_b I used the sibship assignment (Wang 2009) method implemented through the program COLONY2 (Jones & Wang 2010). I explored the relationship between N_b and N_s in ringed seals and environmental parameters in northwestern Hudson Bay. In Chapter 2, I did not find a positive relationship between N_b and N_s with the data available. Ideally, N_b would be compared to the number of breeding-aged individuals (N_a) in a population, but the N_s estimates I used were derived from the total number of individuals in the population, as aerial surveys do not differentiate between age classes. The high number of young-of-the-year seals represent a large proportion of the population in the spring aerial surveys (Appendix B: Figure B2), may explain the lack of an observed positive relationship between N_b and N_s in Chapter 2. I did, however, obtain a rough estimate of the N_b/N_s ratio by taking the mean of all estimates available for each

parameter. The resulting ratio was 0.048 and was similar to published ratios of N_e and the census population size (N_c), that used contemporary estimation methods (Andersen *et al.* 2011; Valtonen *et al.* 2014; Collins *et al.* 2016). The ratio I calculated was also similar to ratios calculated with the sex-ratio equation ($N_e = 4N_m * N_f / (N_m + N_f)$), where N_m and N_f are the number of males and females in the population, for the South American fur seal (*Arctocephalus australis*) (de Oliveira *et al.* 2006). These ratios are less than the meta-analysis published ratios for N_b/N_c of 0.26 (Palstra & Fraser 2012) and 0.11 (Frankham 1995). The estimate of N_e/N_c of 0.36 for ringed seals that used the two-sample temporal method was substantially higher than the ratio we calculated (Valtonen *et al.* 2014). Temporal methods of calculating N_e are able to account for population structure and migration, better than single-sample estimators that apply to a local scale (Waples & England 2011; Gilbert & Whitlock 2015). Overall, N_b and N_s did not change over time. However, N_b declined from 1983 to 1994, during the time before aerial surveys were flown.

In Chapter 3, I examined the relationship between environmental variables that could drive trends in N_b . These variables were the date of spring ice-breakup and fall ice-freezeup, snow depth, and spring rainfall. We found support for relationships between spring rainfall, snow depth, and the date of fall freezeup. The relative strength of snow depth compared to spring rainfall and the date of fall freezeup was not tested, as the sample size for snow depth was limited. I tested the relationship at a series of lags, as N_b 's response to environmental conditions may be delayed due to the time it takes ringed seals to reach reproductive age. The lags that had the strongest support differed for each environmental variable. However, the lags of 5, 6, and 7 years that were associated with the top models reflect the time when the most individuals from a

cohort would be producing offspring. Lags of 5 for spring rainfall and 7 for the date of fall freezeup were still the most likely models when lags from 0-10 were included in model selection (Appendix B: Table B1).

The theoretical expectation is for N_e and the population size (N) to be correlated (Wright 1938; Nunney 2016); however, obtaining the samples needed to calibrate these two parameters is difficult in natural systems. In species that occupy large spatial distributions and have panmictic genetic population structure, estimates of contemporary N_e often result in estimates of the effective number of individuals in the genetic neighbourhood (N_n) that the samples are taken from (Nunney 2016). Unlike N_e , N_n is independent of N and is determined locally by the dispersal biology of the study species (Nunney 2016). Additionally, both N_e and N are challenging to estimate with accuracy in large populations (Luikart *et al.* 2010; Wang 2016; Waples 2016). Waples (2016) points out that in large populations the drift signal is weak, which can lead to point estimates that are much too low or infinite. He noted that very large sample sizes can help to reduce the downward bias of estimates in large populations, but not eliminate them. When N_e is large (e.g., 10 000) and a sample size of 50 with 100 loci is used, approximately half of the estimates with currently available estimators were infinite and the other half were <25% of the true N_e (Waples 2016a). However, when 5000 samples and 7 loci were used, N_e estimates as high as 10 000 with finite confidence intervals were possible to attain in simulations (Macbeth *et al.* 2013). Wang (2016) also showed that the single-sample sibship method could achieve estimates of N_b as large as 30 000 when 100 samples and 20 loci are used.

Meta-analyses of N_e/N_c ratios have indicated that the average ratio is 0.11-0.23 (Frankham 1995; Palstra & Fraser 2012). Therefore, the upper limit of N_e that Wang (2016)

showed to be possible to estimate corresponds to an N ranging from 300 000 to 120 000. It is possible that populations do have a ratio of N_e/N_c closer to 0.5, as theory predicts, but due to limitations of estimating N_e in larger populations the results of meta-analyses have been biased downward. If there are 500 000 (Smith 1975) ringed seals in Hudson Bay, then the expected N_e would be 50 000 to 125 000, which exceeds the magnitude of N_e demonstrated to be achievable with currently available contemporary estimators. If N_e estimates are obtained without estimates of N_c it is possible to bypass calibrating one parameter with the other and use the average ratio between the two. Although, this method is recommended only with extreme caution as the currently available meta-analysis values are derived from studies of fishes, amphibians, and insects and the range of values from individual studies is wide (0.01–0.95) (Palstra & Fraser 2012). The applicability of inferring trends in population size from N_e depends on the size of the population being studied and the availability of samples or potential for collecting them. If the population is <10 000 and a large proportion of samples relative to that population size can be collected, then accurate estimates of N_e are possible. However, in smaller populations the relationship between N_b and N is less predictable than in large populations and can vary temporally (Bernos & Fraser 2016). Adding more loci can increase the accuracy of estimates (Wang 2016), but there is a limit to the number of loci that can be added before pseudo replication begins to occur (Waples & Faulkner 2009). Adding more markers after sibship assignment has successfully occurred can cause a slight decline in accuracy of estimates with the sibship method, but increase accuracy with the linkage disequilibrium method (Waples 2016b). An alternative approach for using N_e has been recommended in situations where mean estimates are erratic and upper confidence intervals are often infinite (Fraser *et al.* 2007; Waples & Do

2010), where the lower confidence interval can be used to monitor as it is more stable and is typically finite (Macbeth *et al.* 2013).

Ringed seal abundance in the southern extent of their range may decline over time due to environmental changes and increases in competitors such as harbour seals (*Phoca vitulina*) or intensified predation by species such as killer whales (*Orcinus orca*) (Kovacs & Lydersen 2008; Higdon & Ferguson 2009; Wassmann *et al.* 2011). The increased occurrence of temperate species may also lead to the introduction of novel pathogens (Kovacs & Lydersen 2008). Shifts in the ecosystems may lead to changes in the abundance of ringed seals through bottom up effects. For example, declines in prey species such as Arctic cod (*Boreogadus saida*) and increases in alternative prey such as capelin (*Mallotus villosus*) and sand lance (*Ammodytes* spp.) may have energetic costs as southern species typically have lower lipid content (Tynan & Demaster 1997; Rose 2005). Additionally, as a trend towards increased temperatures and decreased duration and extent of sea ice occurs, resource extraction and development will become more economically viable in the Arctic (Huntington 2009). Increases in industrial activities such as increased shipping traffic will potentially disrupt habitat and create both chemical and noise pollution (Wilson *et al.* 2014).

If the accuracy of N_b and N_e estimators continues to improve, they may become a very valuable tool for population monitoring in the Arctic, where surveys are expensive and many charismatic and harvested species have experienced declines in recent decades. With currently available methods, inferring population size from N_b or N_e for the purpose of making management decisions should be done with caution (Palstra & Fraser 2012; Bernos & Fraser 2016; Yates *et al.* 2017). Lack of stability of the ratio between parameters and the ability to

estimate N_b or N_e accurately are problematic (Palstra & Fraser 2012; Yates *et al.* 2017).

Although there are not currently quotas assigned to ringed seals in Canada, population estimates inferred from N_b or N_e that underestimated population size could lead to overly stringent conservation measures that negatively affect food security of northerners.

Given that emission scenarios are showing that the effects of climate change will persist (Overland *et al.* 2013), it is crucial to develop and maintain co-management conservation strategies for this species (Laidre *et al.* 2015). With studies showing that arctic marine mammals demonstrate a heterogeneous response to climate change over their distributions and examples of populations with both positive (Stirling *et al.* 2011; Rode *et al.* 2014; George *et al.* 2015) and negative (Simmonds & Isaac 2007; Molnár *et al.* 2010; Ferguson *et al.* 2017) responses to climate change, it is important to continue data collection in different regions so that local effects can be tracked and policies specific to those regions can be developed. Working with indigenous and non-indigenous northerners who are stakeholders and therefore have a vested interest in further conservation of Arctic marine mammals as an economically and culturally important resource, will help to facilitate future data collection and research.

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Appendix A: Laboratory methods and microsatellite analyses

Table A1. PCR recipe

Ingredient	Concentration	Volume	X 10
H ₂ O	X	X	X
5Xbuff	1X	2.0	20
MgCl ₂	1.5mM	0.6	6
dNTP	0.20mM	0.2	2
BSA	0.2µg/µl	1.0	10
F/R	X	X	X
GoTaq	1U	0.2	2
DNA	10-150 ng/µl	1.0	10

Table A2. Locus, dye, and primer concentrations in multiplexes.

Multiplex	Annealing temperature (°C)	Primers	Dye	Concentration (μM)
1	60	Hi-8	VIC	0.40
		Hi-16	FAM	0.30
		Hg6.1	NED	0.16
		Hg6.3	VIC	0.50
2	57	HI-2	PET	0.14
		Lc-28	NED	0.40
		Hg8.10	FAM	0.20
		Lc-6	NED	0.30
3A	55	SGPv11	FAM	0.20
		SGPv9	PET	0.40
		Lc-26	NED	0.60
3B	55	Hg4.2	VIC	0.20
		Hg8.9	FAM	0.40
4	55	SGPV10	VIC	0.40
		Lw-15	NED	0.20
		Lc-18	NED	0.40
		Sex	FAM	0.20
5	48	Hgdii	FAM	0.20
		Hi-15	NED	0.40
		Hi-4	PET	0.80

Table A3. Summary information for 19 microsatellite loci.

Loci	Target sp.	Dye colour	Motif	Source
Hg4.2	<i>Halichoerus grypus</i>	VIC	di-	Allen <i>et al.</i> 1995
Hg6.1	<i>Halichoerus grypus</i>	NED	di-	Allen <i>et al.</i> 1995
Hg6.3	<i>Halichoerus grypus</i>	VIC	di-	Allen <i>et al.</i> 1995
Hg8.10	<i>Halichoerus grypus</i>	FAM	di-	Allen <i>et al.</i> 1995
Hg8.9	<i>Halichoerus grypus</i>	FAM	di-	Allen <i>et al.</i> 1995
Hgdii	<i>Halichoerus grypus</i>	FAM	di-	Allen <i>et al.</i> 1995
Hi15	<i>Lobodontini</i>	NED	di-	Davis <i>et al.</i> 2002
Hi16	<i>Lobodontini</i>	FAM	di-	Davis <i>et al.</i> 2002
Hi-2	<i>Lobodontini</i>	PET	di-	Davis <i>et al.</i> 2002
Hi-4	<i>Lobodontini</i>	PET	di-	Davis <i>et al.</i> 2002
Hi-8	<i>Lobodontini</i>	VIC	di-	Davis <i>et al.</i> 2002
Lc-18	<i>Lobodontini</i>	NED	di-	Davis <i>et al.</i> 2002
Lc-26	<i>Lobodontini</i>	FAM	di-	Davis <i>et al.</i> 2002
Lc-28	<i>Lobodontini</i>	NED	di-	Davis <i>et al.</i> 2002
Lc-6	<i>Lobodontini</i>	NED	di-	Davis <i>et al.</i> 2002
Lw-15	<i>Lobodontini</i>	NED	di-	Davis <i>et al.</i> 2002
SDP 471 (Sex)	<i>P. hispida</i>	FAM		Shaw <i>et al.</i> 2003
SGPv10	<i>Phoca vitulina vitulina</i>	VIC	di-	Goodman 1997
SGPv11	<i>Phoca vitulina vitulina</i>	FAM	di-	Goodman 1997
SGPv9	<i>Phoca vitulina vitulina</i>	PET	di-	Goodman 1997

Table A4. MICROCHECKER (Van Oosterhout *et al.* 2004) results for all samples available from eastern Canada.

Locus	Homozygote excess	Scoring error due to stutter	Long allele dropout	Null alleles
Hi16	No	No	No	No
Hi8	No	No	No	No
Hg6.3	Yes	No	No	Maybe
Hg6.1	Yes	Maybe	No	Maybe
Hg8.10	Yes	No	No	Maybe
Hi4	No	No	No	No
Lc6	No	No	No	No
Hi2	Yes	No	No	Maybe
SGPv11	No	No	No	No
Lc26	Yes	No	No	Maybe
SGPv9	No	No	No	No
Hg8.9	Yes	No	No	Maybe
Hg4.2	Yes	No	No	Maybe
SGPv10	No	No	No	No
Lc18	Yes	No	No	Maybe
Hgdii	Yes	No	No	Maybe
Hi15	Yes	No	No	Maybe
Lc28	Yes	No	No	Maybe
Lw15	Yes	No	No	Maybe

Table A5. Hardy-Weinberg equilibrium (HWE) was assessed for all samples from Arviat, at the 13 loci that were included while estimating N_b . No loci deviated significantly from HWE after a sequential Bonferroni correction.

Locus	HWE	Adjusted α
Lc28a	0.010	0.004
Hi15a	0.027	0.004
SGPv10a	0.058	0.005
Hg63a	0.096	0.005
SGPv11a	0.148	0.006
Hi8a	0.238	0.006
Hg810a	0.254	0.007
Hg89a	0.281	0.008
SGPv9a	0.421	0.010
Hg42a	0.618	0.013
Hi2a	0.740	0.017
Lc6a	0.824	0.025
Hi16a	0.975	0.050

Table A6. Hardy-Weinberg equilibrium (HWE) was assessed for all samples in Hudson Bay, at the 13 loci that were included while estimating N_b . All loci deviated significantly from HWE after sequential Bonferroni correction.

locus	HWE	Adjusted α
Hi8	0.000*	0.004
Hg8.10	0.000*	0.004
Lc6	0.000*	0.005
Hi2	0.000*	0.005
SGPv11	0.000*	0.006
SGPv9	0.000*	0.006
Hg8.9	0.000*	0.007
SGPv10	0.000*	0.008
Hi15	0.000*	0.010
Lc28	0.000*	0.013
Hg6.3	0.007*	0.017
Hi16	0.008*	0.025
Hg4.2	0.010*	0.050

* = significant after sequential Bonferroni correction

Table A7. Linkage disequilibrium assessment was done using GENEPOP (Raymond & Rousset 1995; Rousset 2008). There were four linkages when samples from Arviat, Nunavut were analyzed alone, but no significant linkages when all samples from eastern Canada were included in the analysis.

Locus#1	Locus#2	P-value	Rank	Adjusted α
Lc6	SGPv11	0.000*	1	0.001
Hi2	Hg42	0.000*	2	0.001
Hg810	Hi15	0.000*	3	0.001
Hg89	Hi15	0.000*	4	0.001
Hi16	SGPv10	0.014	5	0.001
Hg810	SGPv11	0.016	6	0.001
SGPv11	SGPv10	0.018	7	0.001
Hi8	Lc28	0.019	8	0.001
Hi8	Hg810	0.029	9	0.001
Hg810	Lc28	0.035	10	0.001
Hi16	Hg810	0.046	11	0.001
Lc6	SGPv10	0.056	12	0.001
Hi16	Hg63	0.086	13	0.001
Hi8	Hi2	0.095	14	0.001
Lc28	Hg42	0.106	15	0.001
Hg63	SGPv10	0.108	16	0.001
Lc6	Hg89	0.109	17	0.001
Lc28	Lc6	0.109	18	0.001
Hi2	Hi15	0.113	19	0.001
Hi8	Hi15	0.114	20	0.001
Hg89	Hg42	0.140	21	0.001
Hi8	SGPv10	0.150	22	0.001
Lc6	Hg42	0.159	23	0.001
Hi16	SGPv11	0.163	24	0.001
Hi8	Hg63	0.197	25	0.001
Hi8	Lc6	0.203	26	0.001
Hg63	Hi15	0.205	27	0.001
Hi16	Lc6	0.255	28	0.001
SGPv9	SGPv10	0.263	29	0.001
SGPv10	Hi15	0.275	30	0.001
Hg810	Lc6	0.276	31	0.001
SGPv9	Hg42	0.284	32	0.001
Lc6	SGPv9	0.288	33	0.001
Hg810	Hg89	0.320	34	0.001
Hi16	Lc28	0.328	35	0.001
Hg63	SGPv11	0.335	36	0.001
Hg63	Lc28	0.353	37	0.001
Hi8	SGPv11	0.383	38	0.001
Hi8	Hg89	0.396	39	0.001
Hi2	Hg89	0.401	40	0.001
Hg89	SGPv10	0.404	41	0.001
Hi2	SGPv10	0.414	42	0.001
Lc6	Hi15	0.440	43	0.001
Lc28	Hg89	0.460	44	0.001
Hi2	SGPv9	0.460	45	0.001
Hi2	SGPv11	0.470	46	0.002

Hg63	Hi2	0.476	47	0.002
Hg63	Hg810	0.514	48	0.002
Hi8	Hg42	0.525	49	0.002
Hi16	Hi15	0.583	50	0.002
Lc28	SGPv10	0.591	51	0.002
Hi16	Hi8	0.599	52	0.002
Hg63	Hg42	0.614	53	0.002
SGPv11	Hi15	0.649	54	0.002
Hg63	Lc6	0.653	55	0.002
SGPv9	Hg89	0.664	56	0.002
Hg810	SGPv10	0.683	57	0.002
Lc28	Hi2	0.697	58	0.002
SGPv9	Hi15	0.778	59	0.003
Hg810	Hg42	0.783	60	0.003
Hg810	SGPv9	0.788	61	0.003
Lc28	SGPv11	0.814	62	0.003
Hi16	Hi2	0.819	63	0.003
Lc28	Hi15	0.833	64	0.003
Hi8	SGPv9	0.848	65	0.004
SGPv11	Hg42	0.849	66	0.004
SGPv11	SGPv9	0.874	67	0.004
Hg63	SGPv9	0.886	68	0.005
Hi16	Hg89	0.913	69	0.005
Lc28	SGPv9	0.920	70	0.006
Hi16	Hg42	0.936	71	0.006
SGPv11	Hg89	0.947	72	0.007
Hi16	SGPv9	0.951	73	0.008
Hg63	Hg89	0.976	74	0.010
Hg810	Hi2	0.990	75	0.013
Lc6	Hi2	0.999	76	0.017
Hg42	SGPv10	1.000	77	0.025
Hg42	Hi15	1.000	78	0.050

*Significant after sequential Bonferroni correction

Table A8. Error rates quantified in PEDANT (Johnson & Haydon 2007).

Locus	Allelic dropout	False alleles
Hi16a	0.003	0.043
Hi8a	0.009	0.025
Hg63a	0.000	0.018
Hg810a	0.021	0.018
Lc28a	0.085	0.040
Lc6a	0.000	0.035
Hi2a	0.013	0.026
SGPv11a	0.015	0.059
SGPv9a	0.000	0.012
Hg89a	0.015	0.214
Hg42a	0.019	0.253
SGPv10a	0.008	0.052
Hi15a	0.000	0.018

Appendix B: Investigation of lag effects and multicollinearity between habitat variables

Table B1. Generalized linear model selection to identify predictors of N_b (n=11). All combinations of the predictive variables including: the date of spring breakup (Break), the date of fall freezeup (Freeze), and the average mm of rainfall that occurred in April and May annually (Rain), aside from Freeze + rain, were compared to determine which models fit the data best with lags 0-10. Sample size (n), Akaike's information criterion corrected for small sample sizes (AICc), delta AICc (Δ AICc), log-likelihood (L), AIC weight (W_i), number of parameters (K).

Rank	Lag	Parameters	n	K	AICc	Δ AICc	W_i	L
1	7	Freeze	11	3	96.2	0.0	0.302	-43.4
2	5	Rain	11	3	96.8	0.7	0.216	-43.7
3	5	Null	11	2	98.3	2.1	0.104	-46.4
4	1	Null	11	2	100.3	4.1	0.039	-47.4
5	6	Rain	11	3	100.5	4.4	0.034	-45.6
6	1	Freeze	11	3	100.7	4.5	0.031	-45.6
7	3	Null	11	2	100.8	4.7	0.030	-47.7
8	2	Null	11	2	100.9	4.8	0.028	-47.7
9	0	Null	11	2	101.3	5.1	0.024	-47.9
10	5	Break	11	3	101.5	5.3	0.021	-46.0
11	4	Null	11	2	101.9	5.8	0.017	-48.2
12	5	Break + Rain	11	4	102.0	5.9	0.016	-43.7
13	5	Freeze	11	3	102.1	5.9	0.016	-46.3
14	7	Null	11	2	102.5	6.4	0.013	-48.5
15	6	Null	11	2	102.5	6.4	0.012	-48.5
16	7	Break	11	3	103.3	7.1	0.009	-46.9
17	1	Break	11	3	103.5	7.3	0.008	-47.0
18	1	Rain	11	3	103.8	7.7	0.007	-47.2
19	4	Rain	11	3	103.9	7.8	0.006	-47.3
20	3	Break	11	3	103.9	7.8	0.006	-47.3
21	2	Rain	11	3	104.2	8.0	0.006	-47.4
22	6	Break + Rain	11	4	104.2	8.1	0.005	-44.8
23	2	Freeze	11	3	104.3	8.2	0.005	-47.5
24	2	Break	11	3	104.5	8.3	0.005	-47.5
25	3	Rain	11	3	104.5	8.3	0.005	-47.5
26	3	Freeze	11	3	104.7	8.6	0.004	-47.7
27	0	Break	11	3	105.1	8.9	0.004	-47.8
28	0	Rain	11	3	105.1	9.0	0.003	-47.8
29	0	Freeze	11	3	105.2	9.0	0.003	-47.9
30	4	Break	11	3	105.2	9.1	0.003	-47.9
31	4	Freeze	11	3	105.6	9.4	0.003	-48.1
32	6	Freeze	11	3	106.3	10.2	0.002	-48.4

33	8	Null	11	2	106.4	10.3	0.002	-50.5
34	7	Rain	11	3	106.4	10.3	0.002	-48.5
35	6	Break	11	3	106.5	10.3	0.002	-48.5
36	7	Break + Rain	11	4	106.6	10.5	0.002	-46.0
37	1	Break + Rain	11	4	107.0	10.8	0.001	-46.2
38	7	Freeze + Break + Rain	11	5	107.1	10.9	0.001	-42.5
39	9	Null	11	2	107.7	11.5	<0.001	-51.1
40	9	Break	11	3	108.0	11.9	<0.001	-49.3
41	0	Null	11	2	108.1	11.9	<0.001	-51.3
42	5	Freeze + Break + Rain	11	5	108.2	12.1	<0.001	-43.1
43	8	Freeze	11	3	109.0	12.9	<0.001	-49.8
44	4	Break + Rain	11	4	109.2	13.0	<0.001	-47.2
45	3	Break + Rain	11	4	109.2	13.0	<0.001	-47.3
46	2	Break + Rain	11	4	109.4	13.2	<0.001	-47.3
47	0	Break + Rain	11	4	109.9	13.8	<0.001	-47.6
48	8	Break	11	3	110.2	14.1	<0.001	-50.4
49	8	Rain	11	3	110.3	14.2	<0.001	-50.4
50	0	Freeze	11	3	110.6	14.5	<0.001	-50.6
51	6	Freeze + Break + Rain	11	5	110.7	14.5	<0.001	-44.3
52	9	Freeze	11	3	110.8	14.6	<0.001	-50.7
53	9	Break + Rain	11	4	110.8	14.7	<0.001	-48.1
54	0	Rain	11	3	110.9	14.7	<0.001	-50.7
55	1	Freeze + Break + Rain	11	5	111.0	14.9	<0.001	-44.5
56	9	Rain	11	3	111.5	15.4	<0.001	-51.1
57	0	Break	11	3	112.0	15.8	<0.001	-51.3
58	2	Freeze + Break + Rain	11	5	114.4	18.2	<0.001	-46.2
59	8	Break + Rain	11	4	115.2	19.0	<0.001	-50.3
60	0	Break + Rain	11	4	116.0	19.9	<0.001	-50.7
61	3	Freeze + Break + Rain	11	5	116.1	19.9	<0.001	-47.0
62	4	Freeze + Break + Rain	11	5	116.4	20.2	<0.001	-47.2
63	0	Freeze + Break + Rain	11	5	117.3	21.1	<0.001	-47.6
64	9	Freeze + Break + Rain	11	5	118.0	21.9	<0.001	-48.0
65	8	Freeze + Break + Rain	11	5	119.6	23.4	<0.001	-48.8

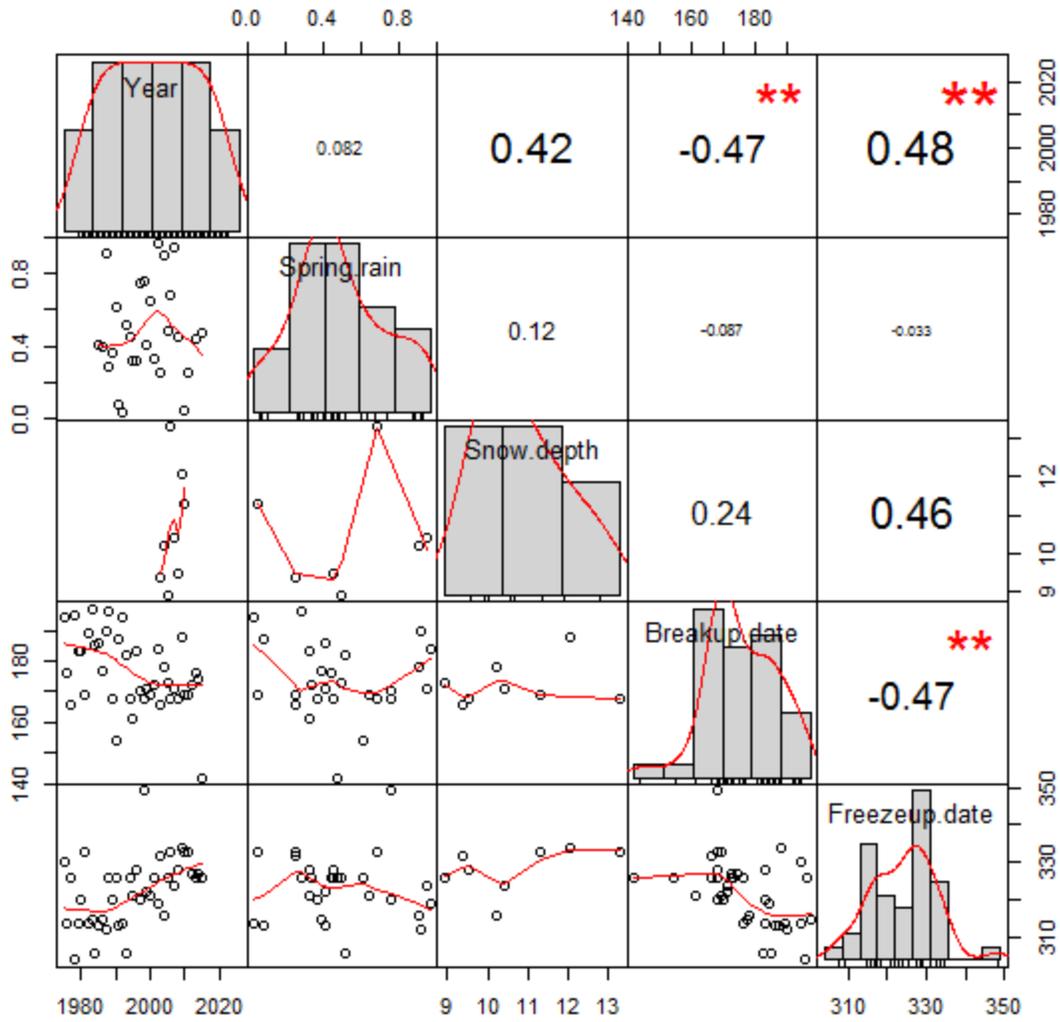


Figure B1. Pearson's correlation matrix examining multicollinearity between habitat variables.

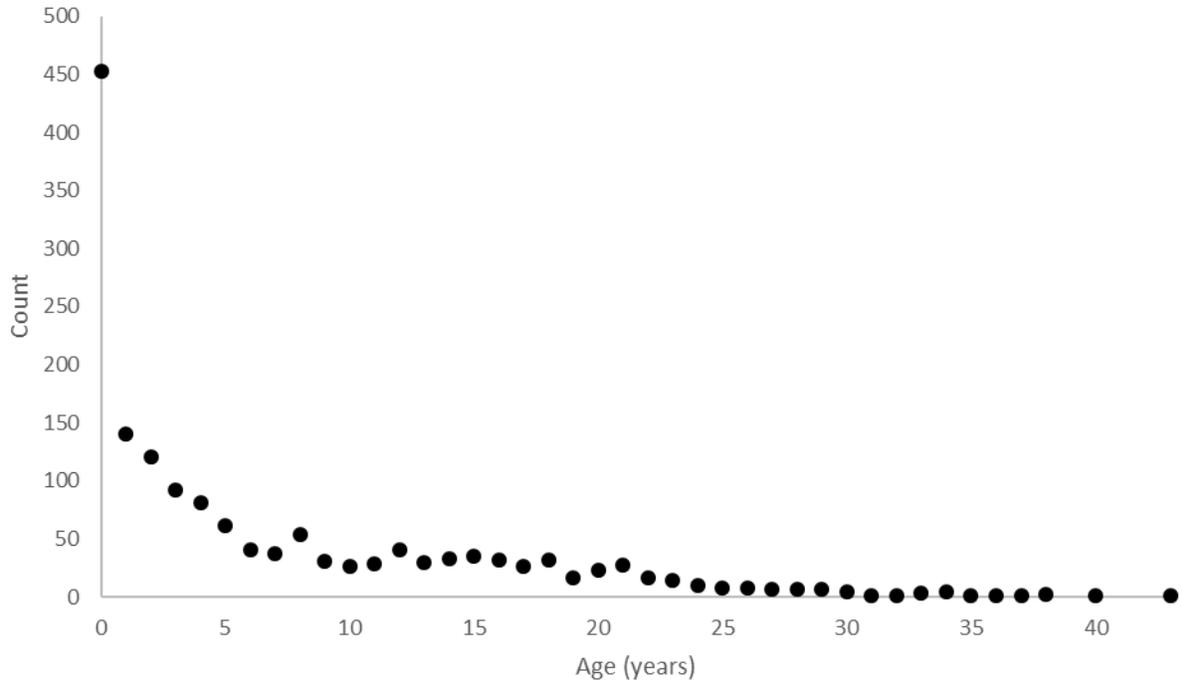


Figure B2. Age distribution of ringed seals (*Pusa hispida*) collected in eastern Canada.

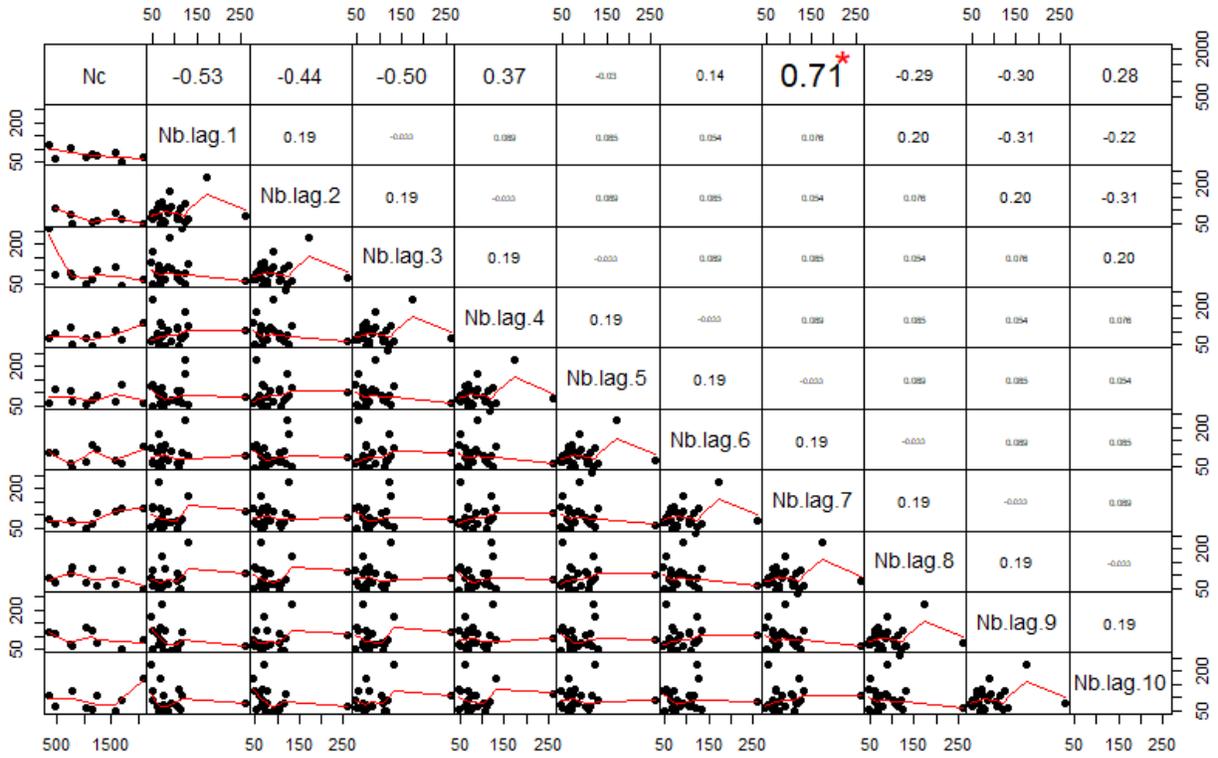


Figure B3. Correlation matrix comparing N_c to N_c with lags of 0 to 10 in N_b .

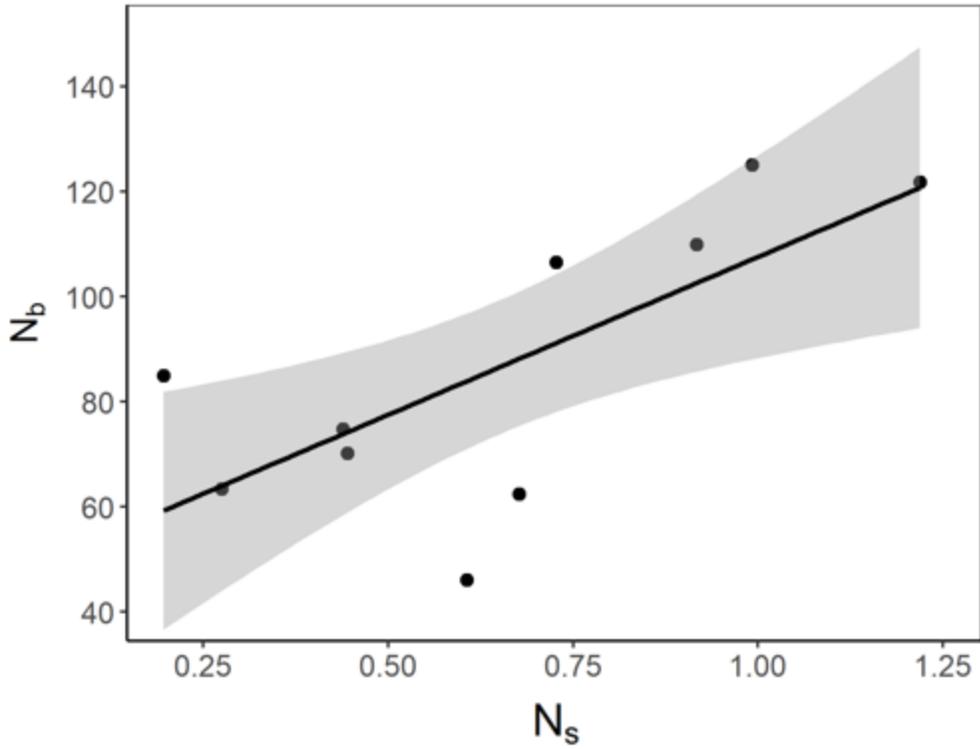


Figure B4. Linear regression between N_b and N_s with a 7-year lag in N_b ($R^2 = 0.505$, $T_9 = 2.854$, $p = 0.021$).