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ASSESSING THE STATUS OF THE ENDANGERED NORTH ATLANTIC RIGHT WHALE USING GENETIC AND DEMOGRAPHIC DATA.

By

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A Thesis

Submitted to the School of Graduate Studies

of

McMaster University

In partial fulfillment of requirements for

the degree of Doctor of Philosophy

in Biology

September, 1999

Demographic and Genetic analysis of the North Atlantic right whale

DOCTOR OF PHILOSOPHY (1999) McMASTER UNIVERSITY

(Department of Biology) Hamilton, Ontario

TITLE: Assessing the status of the endangered North Atlantic right whale using genetic and demographic data.

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SUPERVISOR: Dr. Bradley N. White

NUMBER OF PAGES: xiv, 172

General Abstract

Nineteen years of monitoring the highly endangered North Atlantic right whale (*Eubalaena glacialis*) has led to the photo-identification of 388 individuals of which 283 were alive in 1997. In addition, sighting histories have been collected for 92 reproductive females and the 208 calves they produced from 1980 to 1997. Calf production and individual survivorship are low in the North Atlantic right whale and are notably lower than that observed in a related species, the South Atlantic right whale (*E. australis*). Since intensive monitoring began in 1980 there has been no evidence of population growth in the former species; recent analyses indicate that the population may actually be declining. Two intrinsic factors that may be affecting the reproductive fitness of adults, thereby limiting the ability of this population to increase in size, are inbreeding depression and low levels of genetic variability.

To assess the status of the population, microsatellite markers were developed and used to establish individual-specific genetic profiles for 209 whales. Genetic profiles based on nine microsatellite loci and mitochondrial control region haplotypes were used to examine the population for evidence of inbreeding and to assess the current levels of genetic variability. At the population level, genetic variability was found to be lower than that reported for other large-bodied cetaceans, including the closely-related South Atlantic right whale. No evidence was found to indicate that the low genetic variability is the result of the most recent population bottleneck (19th century). Rather, the loss of

genetic variability appears to have occurred over several hundred years, starting as far back as the 16th century. Any reduction in fitness associated with the loss of genetic variability, including reduced reproductive success and the loss of adaptive potential, may have been affecting this population for over 400 years.

To examine the current population for evidence of inbreeding, the nature of the mating system was identified and used to estimate the effective number of breeders in the population. The individual-specific genetic profiles were used to evaluate relatedness among the 209 whales, including that among breeding pairs. Mating by North Atlantic right whales had been thought to involve dominance polygyny. In fact, most of the calves evaluated in the paternity analysis had different fathers, and the pattern of paternity assignments was most similar to that of random mating (*i.e.*, lottery polygyny or promiscuity). Under random mating, a larger proportion of adults will contribute to the next generation, thereby slowing the rate of loss of genetic variability. Based on a promiscuous mating system, the effective population size is estimated at between 103 and 154, which places the ratio of the census population size to the effective population size within those reported for other large-bodied mammals.

Despite the random nature of the mating system, population structuring was identified between calves born to two groups of females that differ in their use of nursery habitat. Reproductive isolation is incomplete, however, and the differences between the two populations are being progressively lessened through gene flow. The identification of

two reproductively discrete populations suggests the existence of two mating areas for the North Atlantic right whale population. Although mating is not entirely random within the population, no evidence of inbreeding was detected in the population. Mating pairs were no more related than unrelated whales ($R_{xy} = 0.0007 \pm 0.24$ versus -0.035 ± 0.28). While 74% of females produced at least one calf during a period of 17 years, only 55% had multiple birthing events.

Given that genetic variability appears to have been lost over the past 400 years, any consequences of inbreeding would have been expressed historically. The failure of the population to increase in size during the past few hundred years may reflect an overall reduction in the reproductive fitness of females that may be associated with the historic loss of genetic variability. The ability of the population to remain at a stable size (~300) throughout the past 400 years suggests that the current decline in the size of this population is the result of more contemporary influences. The combination of contemporary influences, such as resource shortages and accidental mortality, with the low intrinsic calving rate, appear to be compromising the long-term persistence of this species.

Preface

This thesis is organized in an 'open-faced' format wherein each of the main chapters has been accepted for publication, submitted for publication, or is in preparation for submission to a scientific journal.

Contributions have been made to this thesis from collaborators from the New England Aquarium (Scott Kraus, Moira Brown and Phil Hamilton) and McMaster University (Department of Biology; Cindy Tomlinson, Sobia Malik, and John Hanlon).

Acknowledgements

Where does one start? For some reason, the undertaking of a PhD is the equivalent of an entire (new) life lived. It has been an incredible experience and I have been extremely fortunate throughout the course of This Life to have met some of the most wonderful people imaginable and to have made many lifetime-friends. As amazed as I am to hear myself saying this, these people made Hamilton feel like my home and I thank them for this.

Throughout my academic training, I have been greatly privileged in having a series of mentors who instructed me well (and tirelessly) and, in doing so, facilitated my growth as a researcher. For this, I wish to present my most sincere appreciation and thanks to Drs. John Gee, Richard Wassersug, and Bradley White. I would also like to thank Drs. Lisle Gibbs, Jim Quinn, 'Brain' (no this is not a type-o) Golding, and Susan Dudley for their interest and willingness to provide help. I am indebted to my colleagues at the New England Aquarium for their interest and involvement throughout my PhD: Moira Brown, Phil Hamilton, Amy Knowlton, and Scott Kraus.

I must say, however, that this entire life experience would have been far less interesting overall if not for the comradeship in the lab. Thanks to Brentle Murray for

being so much fun and Sonya 'Never-say-no' Grewall for being the most easy-going person I have ever met. I am privileged in having had the opportunity to know and be friends with all the rest of the folks whom I have known through the lab: Deborah, John, Joyce, Monica, Marvin, Cindy, Aria, and Arkan. I am pleased also at now having the time to spend getting to know some of the 'new' people better: Jen, Leane, Tim, Carla, and Toolika. I also have to mention some people who not only went out of their way to help me and make me laugh, but also made Hamilton feel like home to me. I cannot thank you enough: Pat Hayward, Kathy Greaves, Kathy Macintosh, Hanna Lindeman, Marg Biggs, Barb Reuter, Lilie De Sousa, and Laila Bastedo.

I am now going to be as bold as to say that my happiness and success was inevitable given the friendship and love from minhas irmâs queridos 'Jak-a-linee' Dockray and 'Moka' Fejtek. I can't believe that I am so lucky as to have friends as incredible as you two, you have my complete love and admiration.

Most importantly, I need to thank my parents for their support and encouragement during the past eight years. Simply saying thanks is inadequate, but my sentiments defy words.

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Scope of thesis

This thesis concerns the genetic assessment of the endangered North Atlantic right whale. The small size of the North Atlantic right whale population (~300) and its failure to increase in number despite over 60 years of protection, make this one of the most highly endangered of all large-bodied cetaceans. By the 19th century, over 700 years of commercial harvesting had severely reduced the size of this population from tens of thousands of individuals to several hundred animals. The failure of the population to increase in number is, in part, the consequence of the low rate of calf production by females. Resource shortages, human-caused mortality, and inbreeding depression have all been suggested as possible factors influencing both the low calf production by females and low individual survivorship. The focus of this thesis is on assessing the importance of inbreeding and low genetic variability on the low realized calf production in the North Atlantic right whale. The relative importance of historic and contemporary influences on the low calf production in this small population were considered with regard to bottleneck events and the nature of the mating system.

This thesis is presented as a sequence of individual papers that cover the development of microsatellite markers for establishing individual-specific genetic profiles and their use in the study of the endangered North Atlantic right whale. Chapter one provides an overview of key concepts in population genetics with particular emphasis on the use of molecular genetic data in the study of the biology of cetaceans. Chapter two is written as a 'Primer note' and briefly outlines the development of the microsatellite

markers that I developed and used to assess the North Atlantic right whale. Chapter three is formatted for submission as a short report. This chapter includes a broad range of analytical approaches that illustrate how individual-specific genetic profiles may be used to identify the existence of unknown animals and population structure. Through the use of genetic profiles, I was able to identify characteristics of the North Atlantic right whale that were not apparent through behavioural monitoring alone. Chapter four and five are both formatted for submission as full papers. These chapters focus on assessing the historic and contemporary levels of genetic variability in the population. In the General Discussion, the implications of the timing of the loss of genetic variability that was identified in chapter four and the nature of the mating system (chapter five) are considered with regard to the low calf production and population growth rate observed in this species.

Chapter one

General Introduction and Literature Review

Populations with high levels of genetic variation have a greater potential to respond to changes in their environment over evolutionary time scales (Lande 1988). Consequently, maintaining existing levels of genetic variation has become an important conservation objective (Frankel and Soule 1981; Soule 1986). Random fluctuations in gene frequencies over time will affect small populations more than large populations. As a result, many threatened and endangered species are subject to losses of genetic diversity (*i.e.*, random genetic drift; Crow and Kimura 1970). Since populations with low levels of genetic variability have a limited range of responses to changes in their environment, small or isolated populations are more vulnerable to both inbreeding depression and extinction than are large populations (Soule 1986, 1987; Lynch *et al.* 1995; Frankham 1998). Over ecological time scales, the loss of genetic variability will increase the incidence of homozygosity, exacerbating the negative effects of inbreeding (Frankel and Soule 1981; Charlesworth and Charlesworth 1987; Thornhill 1993; Keller *et al.* 1994; Frankham 1995). Large populations that undergo a sudden reduction in number will lose genetic variability; this loss may compromise the fitness of a population through increased homozygosity, which leads to the expression of deleterious recessive alleles, or the loss of heterotic effects (Allendorf 1986; Lacy 1987). Such consequences of reduced genetic variability have been shown to reduce individual survivorship and reproductive fitness in domestic and captive populations (Falconer 1981; Ralls *et al.* 1988; Thornhill 1993). Most of the evidence for inbreeding depression comes from studies of captive populations (Ralls *et al.* 1979, 1988; Lacy *et al.* 1993; Lynch *et al.* 1995). Reduced fitness effects associated with low levels of genetic diversity have been documented in

some natural populations (Wildt *et al.* 1987; Thornhill 1993; Keller *et al.* 1994).

The rate at which genetic variability is lost or gained is a function of the number of successful breeders (N_e) in a population (Nei 1987). Some adults reproduce more successfully than others, consequently, the effective population size (N_e; Nei 1987) is smaller than the adult population size (Frankham 1995). The magnitude of the difference between the number of successful breeders in the population and the census population size (N) depends on the variance in mating success among adults within a population. Variance in reproductive success may be high if a population has a skewed sex ratio or if there is a large variance in progeny production among breeders (Wright 1931; Waples 1991; Nunney and Elam 1994; Rockwell and Barrowclough 1995). Non-random mating strategies result in high variance in breeding success and this, in turn, will reduce the effective number of breeders in a population. Small population size and non-random mating will exacerbate genetic drift, thereby increasing the likelihood of allele loss and fixation (Crow and Denniston 1988; Harris and Allendorf 1989). Directly calculating the precise N_e of a population requires that information on individual lifetime reproductive fitness is available. As a result, individuals must be identifiable and their reproductive activities must be monitored throughout their lifetime. Individual-specific reproductive histories are logistically difficult to obtain for natural populations, consequently, N_e is usually estimated using indirect methods. For example, allele frequency data for a population may be used to estimate N_e indirectly using the observed changes in population allele frequencies or heterozygosity over time (Nei 1987; Waples 1991).

If the size of a population fluctuates over time, the best approximation of the long-

term N_e is the harmonic mean (Vucetich *et al.* 1997). As a result, dramatic reductions in the size of a population, even when followed by population expansion, accelerate the loss of genetic diversity. Because it is the rare alleles that tend to be lost during such a bottleneck event, these events have little effect on short-term changes in heterozygosity (Luikart and Cornuet 1998; Luikart *et al.* 1998). Alleles are lost more rapidly than heterozygosity during a bottleneck event, consequently, populations that have recently undergone a bottleneck will have a disproportionate number of loci with a heterozygote excess than would be expected by chance (Luikart *et al.* 1998). This heterozygote excess is transient and is only detectable until a new equilibrium is reached between genetic drift and mutation (0.2-4 N_e generations). If a post-bottleneck population is small, the proportion of breeding events between individuals with alleles that are identical-by-descent will increase. If a bottleneck occurs over a protracted period of time, the expression of deleterious recessive alleles will occur gradually and this may actually benefit a population by reducing its genetic load (see Nei 1987).

In order to evaluate the extent to which inbreeding will affect individual fitness, the relationship among breeders must be identified and some measure of their reproductive fitness must be known (Haig *et al.* 1990; Packer *et al.* 1991; Hedrick and Miller 1992; Lacy *et al.* 1996). Although few pedigrees are known for natural populations (but see Pemberton *et al.* 1991; Coulson *et al.* 1998) the relationships among individuals may be inferred through the use of genetic markers (Queller and Goodnight 1989; Piper and Rabenold 1992; Blouin *et al.* 1996). By looking at the proportion of alleles shared at individual loci, markers with Mendelian inheritance patterns may be used to identify

relationships among individuals. The capacity to differentiate among individuals related as first or second order relatives depends on the number and frequency of alleles in the population (Chakraborty and Jin 1993); highly polymorphic markers are required for such studies. If a population has low levels of variation, the power of such relatedness studies may be improved by studying a large number of loci (Chakraborty and Jin 1993; Blouin *et al.* 1996).

Populations that have experienced bottleneck events are expected to have low levels of genetic variability; genetic analyses of relatedness and inbreeding will require the identification of highly variable molecular markers. Microsatellites are a particularly useful class of genetic markers for such studies because they are highly polymorphic and are distributed throughout the genome (Tautz 1989; Bruford and Wayne 1993; Blouin *et al.* 1996). Moreover, as microsatellites are small tandemly repeated di-, tri-, or tetra-nucleotide repeats (100-400 bp), they may be amplified using polymerase chain reaction (PCR) technology (Weber and May 1989). The small amounts of DNA required for PCR analyses are especially practical in the study of small populations, particularly if a species is rare or endangered. Microsatellite-based profiles also have broad applications and may be used to establish individual identities (Balding and Donnelly 1995; Palsbøll *et al.* 1997; Waser and Strobek 1998), analyze social group structure and mating systems (Amos *et al.* 1993; Morin *et al.* 1993; Blouin *et al.* 1996; Clapham and Palsbøll 1997), evaluate levels of genetic diversity at the individual and population level (Taylor *et al.* 1994; Houlden *et al.* 1996; Hoelzel *et al.* 1998), identify structuring within populations

(Paetkau *et al.* 1995; Berubé *et al.* 1998), and examine evolutionary relationships among species (Bowcock *et al.* 1994; Paetkau *et al.* 1997).

The Mendelian inheritance pattern of microsatellites make them well suited for use in relatedness studies and parentage analysis, since the proportion of shared alleles at these loci may be predicted based on probabilities (Bruford and Wayne 1993; Blouin *et al.* 1996). The principle for such an analysis of relatedness or kinship is based on the 'identity-by-descent' of alleles between individuals (Ritland 1996). Individuals that are more closely related to each other will share a larger proportion of alleles than will unrelated individuals. The degree of relatedness between individuals may be estimated using one of two approaches: (1) Queller and Goodnight's (1989) unbiased estimate that weights allele matches between two individuals using the allele frequency in the population (R_{xy}) or (2) by tabulating the number of matching alleles at each locus to estimate the average number of matches between two individuals (M_{xy}; Blouin *et al.* 1996). Although both methods provide similar relatedness estimations, the R_{xy} estimate makes use of allele frequency data, giving it a greater ability to discriminate among related individuals (Blouin *et al.* 1996).

Exclusionary principles used alone or in combination with likelihood analysis allow hypotheses about paternity and mating behaviours to be tested (Hughes 1998; Marshall *et al.* 1998). In some cases, such studies have demonstrated that traditional ideas about mating systems are not always upheld by the genetic relationships (eg., Gibbs *et al.* 1990; Avise 1994). The power to assign paternity will, however, depend on the number of alleles at the locus, the proportion of the potential sires sampled, and the proportion of

first-order relatives that may be competing for matings (Double *et al.* 1997; Marshall *et al.* 1998). Double *et al.* (1997) report, for example, that when father-son pairs are competing for matings, over eight loci with 10 alleles are necessary for an exclusion probability > 90% when the mother is known.

Application of Microsatellites in Population Biology

In small or endangered populations, non-random mating and random genetic drift may have a large effect on the genotype and allele frequencies in a population. Deviations in genotype frequencies from Hardy-Weinberg Equilibrium expectations (HWE) in a population may be evaluated using observed allele frequencies (Nei 1987). If mating is random, a population is large, mutation and migration are negligible, and no selection is affecting the markers under study, alleles will unite according to their frequency in a population. Because population studies generally focus on ecological time scales, and microsatellites are non-coding, genotypes will only deviate from HWE if null alleles are present, inbreeding is occurring, the population is structured, migration is occurring, or mating is non-random (Bruford and Wayne 1993). Once populations have been identified as being out of HWE, further study may be used to identify those factors that may be influencing the observed deviation. The analysis of microsatellite data in Atlantic walrus (*Odobenus rosmarus rosmarus*), for example, identified deviations from HWE that led to the identification of four geographically distinct populations (Andersen *et al.* 1998). Additional analysis using mitochondrial DNA (mtDNA) showed that the observed structuring among populations was due to limited dispersal of females among the

populations. Analysis of the genetic structure between seemingly distinct populations has also led to the identification of extensive gene flow between populations that were believed to be geographically separated. Paetkau *et al.* (1995), for example, found that populations of Alaskan brown bears (*Ursus middendorffi*) occupying distant island archipelagos and mainland habitats had levels of gene flow that effectively homogenized these populations.

Microsatellites have also been used to assess genetic changes within populations, detect recent population bottlenecks, estimate N_e, and assess levels of inbreeding (Waples 1989, 1991; Frankham 1998; Luikart and Cornuet 1998). Statistical methods are also available for identifying the occurrence and relative timing of bottleneck events for a period of 0.2-4N_e generations (Luikart and Cornuet 1998). Since populations that have undergone a recent, severe bottleneck event may be experiencing an elevated risk of inbreeding depression, it is important that conservationists identify and evaluate the management priorities for these populations. In the case of the northern hairy-nosed wombat (*Lasiorhinus krefftii*), a population bottleneck that is thought to have reduced the N_e to ~10 within the past 120 years caused a 41% loss of heterozygosity (Taylor *et al.* 1994). Despite having low levels of genetic variability, the population has not only persisted but has increased numerically since the bottleneck event. This observation suggests that this population may be able to recover without the introduction of animals from other, distant populations.

Individual-specific genetic profiles are also useful for evaluating relatedness within social groups, estimating population abundance, and for identifying the population to

which individuals belong (Bruford and Wayne 1993; see Waser and Strobek 1998). For example, the analysis of 3,060 humpback whale (*Megaptera novaeangliae*) samples established that they represented 2,368 animals (Palsbøll *et al.* 1997). The sampling locations and recapture data were then used to identify migratory movements and to provide information on the exchange of individuals across habitat areas. Individuals may also be assigned as members of one or another population using likelihood principles that contrast the allele frequencies in an individual to two possible source populations. A benefit of being able to assign individuals as members of populations based solely on their allelic composition is that the identity of unknown individuals may be established without the need to tag the animals.

Studies of relatedness among social groups have also benefitted from the development of individual-specific genetic profiles (Valsecchi and Amos 1996; Palsbøll *et al.* 1997). For species like cetaceans, difficulties in recognizing individuals and tracking their behaviour (Larsen *et al.* 1996; Palsbøll *et al.* 1997) may be overcome through kinship analysis using genetic data. Microsatellite primers developed for long-finned pilot whales (*Globicephala melaena*) were used to examine the relationship among cohesive social groups (Amos *et al.* 1993). The cohesion and helping behaviour of members of these groups was found to be influenced by kinship, suggesting that individuals are obtaining inclusive fitness benefits from being members of these social (kin) groups (see Queller and Goodnight 1989). Relatedness and parentage analyses may be used to identify the mating system of a species even when individual identities are

unknown (Clapham and Palsbøll 1997). Moreover, if mating pairs can be identified, direct assessments of the levels of inbreeding in a population will be possible (Frankham 1995; Blouin *et al.* 1996; see also Ritland 1996).

Cetacean population genetics

Extensive ranging behaviours and the marine lifestyle of cetaceans make individual identification, behavioural studies, and stock identification logistically difficult (Amos *et al.* 1991). The application of molecular-genetic techniques to the study of cetaceans has advanced our understanding of stock boundaries, gene flow, social group structure, mating systems, and phylogeny (Amos *et al.* 1991; Hoelzel 1994; Milinkovitch *et al.* 1994; Larsen *et al.* 1996; Clapham and Palsbøll 1997; Hoelzel *et al.* 1998; Lyrholm and Gyllensten 1998). The molecular marker selected for use will depend on the nature of the question. Studies of mitochondrial (mt) DNA diversity and structuring allow phylogenetic comparisons to be made, and can provide information on behavioural substructuring that is maternally directed (Milinkovitch *et al.* 1994; Baker *et al.* 1998; Lyrholm and Gyllensten 1998; Malik *et al.* In Press). The analysis of nuclear DNA provides information both at the individual and population level and may be used to establish individual identifications, kinship, parentage, levels of genetic diversity, and to estimate levels of gene flow (see Bruford and Wayne 1993).

Phylogenetic assessments of cetacean taxa based on morphological features have proven difficult due to the highly modified traits within this taxa (Milinkovitch *et al.*

1994). The classification of cetacean taxa is not only complicated by difficulties in morphologically-based studies, but also inconsistencies between genetic and morphological analyses. Árnason *et al.* (1993), for example, found that the level of genetic differences between two populations within a single genus (*Balaenoptera* spp.; minke whales) was of a similar magnitude to that observed between two families of baleen whales (Balaenopteridae and Eschrichtiidae). In a study of the phylogeny of cetacean taxa, Milinkovitch *et al.* (1994) compared ribosomal and cytochrome b sequences (mtDNA) for all major cetacean taxa. Although most of the broad classifications matched those based on morphology, one group of toothed whales (sperm whales) was found to be more related to the baleen whales (suborder Mysticeti) than to other toothed whales (suborder Odontoceti; Milinkovitch *et al.* 1994). To resolve these discrepancies, multiple genetic loci and morphological analyses must be examined both independently and collectively.

MtDNA has also proven useful for identifying behavioural differences associated with maternal lineages (Schaeff *et al.* 1993; Baker *et al.* 1998; Lyrholm and Gyllensten 1998). Structuring of mtDNA haplotypes has been observed in association with differential use of feeding or reproductive grounds by members of a panmictic population (Larsen *et al.* 1996). In their study of the North Atlantic humpback whale, Larsen *et al.* (1996) found evidence of genetic exchange between eastern and western populations but noted the existence of two distinct matrilineal groups. The structured distribution of these mtDNA haplotypes was partitioned according to site fidelity to specific feeding grounds. MtDNA structuring has also been observed between proximal populations of beluga

whales (Brennin *et al.* 1997; Brown Gladden *et al.* 1997). In this instance, genetic differences between whales in the eastern Hudson Bay and the western and central Arctic indicate that despite their relative proximity to each other, these groups represent geographically distinct populations.

Although markers within the nuclear genome may also be used to assess population structuring and phylogenetic relationships, the Mendelian inheritance pattern of these markers make them especially useful in the study of mating systems, parentage, effective population size, relatedness, gene flow, and genetic drift (see review by Bruford and Wayne 1993). In an early analysis of social group structure, Amos *et al.* (1993) found that pilot whale pods consisted of extended family groups. The failure of adult males to disperse from their natal pod, despite the fact that they do not breed within the group, suggested that individuals derive inclusive fitness benefits from these long-term associations. While long-term behavioural studies of cetaceans have provided extensive data on the migration and behaviour of some species (Knowlton *et al.* 1992; Clapham and Palsbøll 1997), difficulties in observing and confirming copulation events has limited our ability to identify the nature of their mating systems. Nevertheless, substantial insights into mating strategies are possible with a limited number of well selected DNA samples. Clapham and Palsbøll (1997) used microsatellite-based genetic profiles to assess the number of males responsible for siring calves born to three female humpback whales. They were able to show that the three calves had each been sired by different fathers, thereby suggesting that mating may be promiscuous in humpback whales. Molecular-

genetic analysis of cetacean populations, particularly when combined with demographic data, has the power to provide a wealth of information on individual behaviours and the structure of cetacean populations.

The right whale

Right whales (*Eubalaena* spp.) are large-bodied baleen whales that occur globally as three populations: one in the North Atlantic, another in the North Pacific, and the third in the Southern Hemisphere. Until recently, the populations were often recognized as three subspecies: the North Atlantic population (*Eubalaena glacialis glacialis*), Southern population (*E. g. australis*), and North Pacific population (*E. g. japonica*). The inability to identify consistent differences between specimens from the different populations has resulted in the reclassification of the populations into two subspecies: *Balaena glacialis glacialis* in the Northern Hemisphere and *B. g. australis* in the Southern Hemisphere. In the Atlantic ocean, there is no overlap in the range of the two taxa and hybridisation is unlikely, as the two subspecies are six months out of synchrony in the timing of reproduction (Schevill 1986; Brown 1994).

Both species of the right whale have similar body size, and overall morphological features, and share similarities in their breeding biology (Schevill *et al.* 1986; Winn *et al.* 1986; Knowlton *et al.* 1994). Female North and South Atlantic right whales are iteroparous and have similar ages at first parturitition (9 versus 7.6 years, respectively) and average calving intervals (3.3 versus 3.7 years, respectively; Payne *et al.* 1990; Knowlton *et al.* 1994; Burnell 1998). Unlike female South Atlantic right whales,

however, no female North Atlantic right whale has been observed to shorten the calving interval due to the early death of a neonate (see Burnell 1998; NEAq catalogue). Genetic differences exist between the two species at mitochondrial loci (Malik *et al.* In Press); key differences currently exist in the size of their populations and their respective growth rates. The South Atlantic right whale population numbers in the thousands, and is increasing at a rate of *ca.* 1.075 per year (Best and Underhill 1990; Payne *et al.* 1990), while the North Atlantic right whale population consists of around 283 individuals and has a declining growth rate (0.976 per year; Caswell *et al.* 1999).

Status of the North Atlantic right whale

The North Atlantic right whale is a highly endangered species that has been reduced from its former distribution throughout the North Atlantic ocean to a remnant population in the western North Atlantic (Reeves and Mitchell 1986a,b; Aguilar 1986). The history of exploitation extends back to the 11th century when Basque whalers began hunting the species in the eastern part of its range (Aguilar 1986). The intensity of these commercial harvests dramatically reduced the size of the eastern population by the end of the 16th century. Concurrent with the slowing of whaling in the east, whaling activities intensified in the western North Atlantic as Basque, British, Dutch, and Danish whaling efforts expanded. By the 18th century, the western population was already showing signs of depletion (Reeves and Mitchell 1986a,b) and by the end of the 19th century, the North Atlantic right whale fishery had reached commercial extinction.

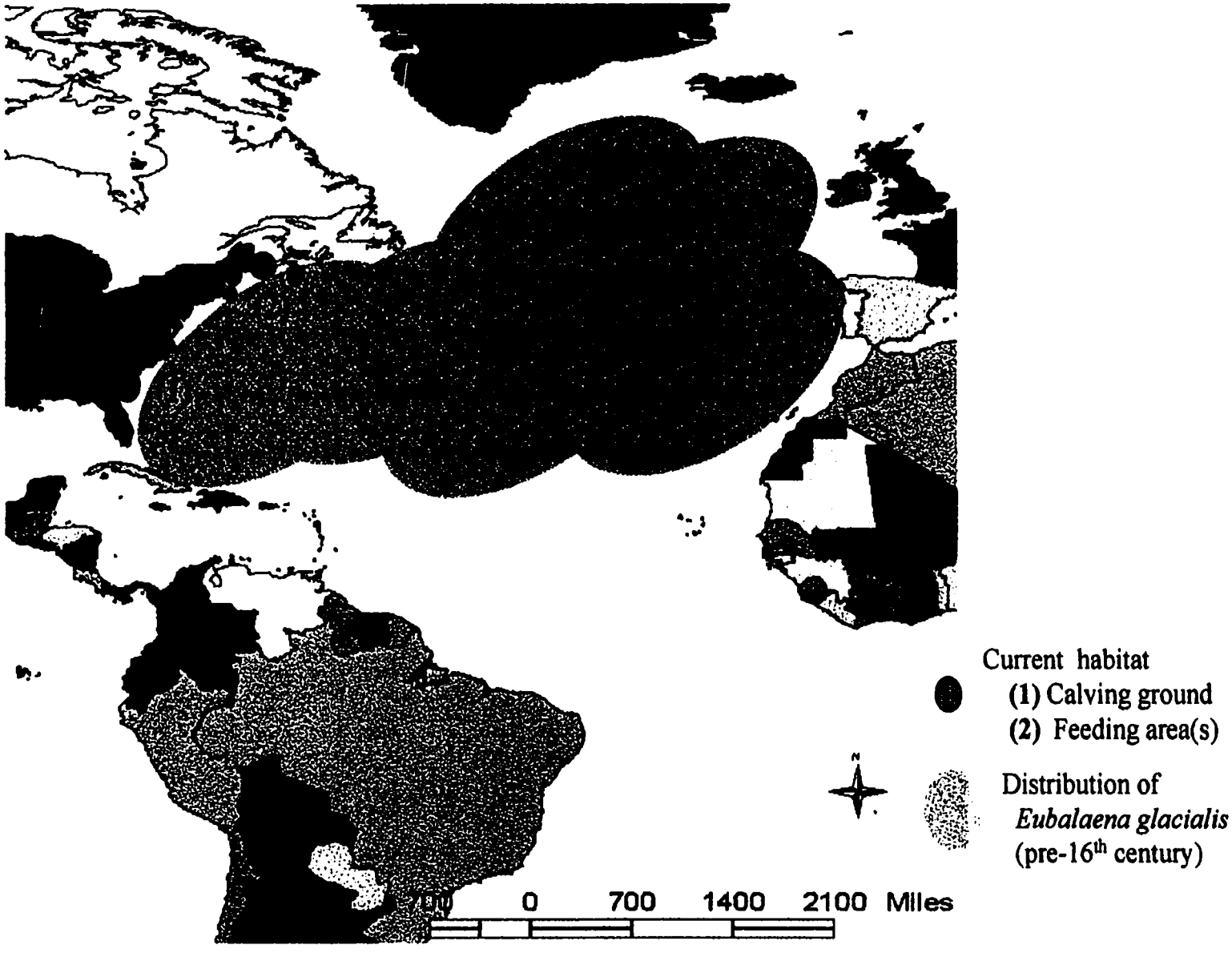
The remaining population of right whales was protected in 1935, however, photo-

identification records suggest that the population size has changed little since this time (Knowlton *et al.* 1994). Although the population size appears to have been stable at *ca.* 300 individuals throughout the past several decades, and recent population modelling suggests that the growth rate for the population was positive in the 1980s (1.053 per year), survivorship seems to have dropped since that time. The current growth rate for the population is now estimated at a declining level (0.976 per year) and the population is expected to face extinction within the next 200 years unless survivorship increases (Caswell *et al.* 1999). Further complicating the growth of this population is the limited reproductive success by adult females (Knowlton *et al.* 1994). The failure of many females to produce more than one calf, and the low survivorship among whales, have been attributed to food shortages (Kenney *et al.* 1986), human-related mortalities (Kraus 1990), and reduced survivorship related to low genetic variability or inbreeding depression (Schaeff *et al.* 1997).

Natural History of the North Atlantic right whale

Almost 20 years of photo-identification records and annual monitoring have identified five habitats that are used seasonally by members of the population (Kraus *et al.* 1986; Knowlton *et al.* 1994; Brown *et al.* 1994). Areas of importance for feeding on zooplankton are those that are occupied by right whales during the summer months, including the Great South Channel, Cape Cod and Massachusetts Bays, and the Bay of Fundy (Kenney *et al.* 1986; Figure 1-1). Although the identity of mother-calf pairs and the reproductive histories of many of the breeding females are known (NEAq Catalogue),

Figure 1-1. Location of identified habitat areas used by western North Atlantic right whales. Seasonal habitats presently used by western North Atlantic right whales include: feeding grounds (Massachusetts Bay, Great South Channel, Roseway Basin), feeding and nursery habitat (Bay of Fundy), and the calving area around Savannah, Georgia and Cape Canaveral, Florida (Southeastern coast of the U.S.A).


Current habitat
(1) Calving ground
(2) Feeding area(s)
N
Distribution of
Eubalaena glacialis
(pre-16th century)
700
0
700
1400
2100 Miles

little is known about reproduction or mating in the North Atlantic right whale. Calf production is known to be synchronous, with breeding females giving birth to their calves during the winter months, and gestation has been estimated at *ca.* 10-12 months (Best 1994; Knowlton *et al.* 1994). Apparent breeding aggregations involving a focal female and multiple males (Surface Active Groups) have been observed throughout the year (Winn *et al.* 1986; Kraus 1991). These SAGs are thought to represent mating groups wherein males compete for positions alongside the female (alpha positions). However, because SAG activity has been observed throughout the range of this species, the timing and location of mating activities remain unknown. If gestation is 12 months (Best 1994) the synchronous calving during the winter months (NEAq catalogue) would indicate that mating also occurs between January and March when the whales are in the southern part of their distribution.

Breeding aggregations and mother-calf pairs represent the only known social interactions between North Atlantic right whales (Lockyer 1984; Oldfield 1988). Calves remain with their mother for approximately one year, during which time they migrate from their place of birth to a summer feeding/nursery area (Kraus 1991). Calving takes place in the southeastern United States off the coasts of Georgia and Florida during the winter months and most of the females that are known to have produced calves have been observed in these waters. Although all females in the population may utilize a common calving ground (but see Watkins and Schevill 1982), mothers differ in where they take their calves when they leave the calving area (Schaeff *et al.* 1993; Malik *et al.* 1999). The majority of the mother-calf pairs migrate north during the spring and are subsequently

identified in the Bay of Fundy during the summer months (Kraus 1991). Another group of females have never been identified with calves in the Bay of Fundy and their whereabouts during the summer months remains unknown (Schaeff *et al.* 1993; Malik *et al.* 1999). Mitochondrial structuring between the two groups suggests that the nursery habitat used by females is selected through culturally-mediated philopatry. It is unknown whether or not this behavioural difference among the females reflects reproductive isolation between subgroups in this population.

Research Objectives and Statement of Hypotheses

The objectives of the present study were to identify polymorphic microsatellite loci in the North Atlantic right whale and use these to evaluate the levels of genetic variability in the population and assess the mating strategy through paternity analysis. The rate at which genetic diversity has been lost from this population and the occurrence of inbreeding were then considered with regard to the low realized calf production. These data were then used to estimate the current and historic effective population size for the North Atlantic right whale.

Hypotheses:

(I) The low reproductive performance in the North Atlantic right whale is a result of a recent loss of genetic variability (*i.e.*, 19th century).

(II) Mating by North Atlantic right whales is by dominance polygny: the

effective population size is smaller than that expected under random mating.

Literature cited

Aguilar A (1986) A review of old Basque whaling and its effects on the right whales (*Eubalaena glacialis*) of the North Atlantic. Report of the International Whaling Commission, Special Issue 10, 191-200.

Allendorf FW (1986) Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology, 5, 181-190.

Amos W, Barrett J, Dover GA (1991) Breeding behaviour of pilot whales revealed by DNA fingerprinting. Heredity, 67, 49-55.

Amos W, Schlotterer C, Tautz D (1993) Social structure of pilot whales revealed by analytical DNA profiling. Science, 260, 670-672.

Åndersen LW, Born EW, Gjertz I, Wiig SO, Holm LE, Bendixen C (1998) Population structure and gene flow of the Atlantic walrus (*Odobenus rosmarus rosmarus*) in the eastern Atlantic Arctic based on mitochondrial DNA and microsatellite variation. Molecular Ecology, 7, 1323-1336.

Arnason U, Gullberg A, Widegren B (1993) Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. Molecular Biology

and Evolution, 10, 960-970.

Avise JC (1994) Natural history of genetic markers. Chapman and Hall, New York.

Baker CS, Medrano-Gonzalez L, Calambokidis J, Perry A, Pichler F, Rosenbaum H, Straley JM, Urban-Ramirez J, Yamaguchi M, Von Ziegesar O (1998) Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. Molecular Ecology, 7, 695-707.

Balding DJ, Donnelly P (1995) Inferring identity from DNA profile evidence. Proceedings of the National Academy of Science, USA, 92, 11741-11745.

Berubé M, Aguilar A, Dendanto D, Larsen SF, Notarbartolo di Sciara G, Sears R, Sigurjønsson J, Urban-R J, Palsbøll PJ (1998) Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. Molecular Ecology, 7, 585-599.

Best PB (1994) Seasonality of reproduction and the length of gestation in southern right whales (*Eubalaena australis*). Journal of Zoology (London), 232, 175-189.

Best PB, Underhill LG (1990) Estimating population size in southern right whales

(*Eubalaena australis*) using naturally marked animals. Report of the International Whaling Commission, Special Issue 12, 183-190.

Blouin MS, Parsons M, Lacaille V, Lotz S (1996) Use of microsatellite loci to classify individuals by relatedness. Molecular Ecology, 5, 393-401.

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. Nature, 368, 455-457.

Brennin R, Murray BW, Friesen MK, Maiers LD, Clayton JW, White BN (1997) Population genetic structure of beluga whales (Delphinapterus leucas): mitochondrial DNA sequence variation within and among North American populations. Canadian Journal of Zoology, 75, 795-802.

Brown MW (1994) Population structure and seasonal distribution of right whales, *Eubalaena glacialis*, in the western North Atlantic. PhD. Thesis, McMaster University.

Brown MW, Kraus SD, Gaskin DE, White BN (1994) Sexual composition and analysis of reproductive females in the North Atlantic right whale, *Eubalaena glacialis*, population. Marine Mammal Science, 10, 253-265.

Brown Gladden JG, Ferguson MM, Clayton JW (1997) Matriarchal genetic population structure of North American beluga whales *Delphinapterus leucas* (Cetacea: Monodontidae). Molecular Ecology, 6, 1033-1046.

Bruford MW, Wayne RK (1993) Microsatellites and their application to population genetic studies. Current Opinions in Genetics and Development, 3, 937-943.

Burnell SR (1998) Aspects of the reproductive biology and behavioural ecology of right whales off Australia. Proceedings of the International Whaling Commission. Cambridge U.K. Doc. SC/M98/RW19.

Caswell H, Fujiwara M, Brault S (1999) Declining survival probability threatens the North Atlantic right whale. Proceedings of the National Academy of Science, USA, 96, 3308-3313.

Chakraborty RM, Jin L (1993) Determination of relatedness between individuals using DNA fingerprinting. Human Biology, 65, 875-895.

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics, 18, 237-268.

Clapham PJ, Palsbøll P (1997) Molecular analysis of paternity shows promiscuous

mating in female humpback whales (*Megaptera novaeangliae*, Borowski). Proceedings of the National Academy of Science USA, 93, 95-98.

Coulson TN, Pemberton JM, Albon SD, Beaumont M, Marshall TC, Slate J, Guinness FE, Clutton-Brock TH (1998) Microsatellites reveal heterosis in red deer. Proceedings of the Royal Society, London, 265, 489-495.

Crow JF, Denniston C (1988) Inbreeding and variance effective population numbers. Evolution, 42, 482-495.

Crow JF, Kimura M (1970) An Introduction to Population Genetics Theory. Burgess, Minneapolis.

Double MC, Cockburn A, Barry SC, Smouse PE (1997) Exclusion probabilities for single-locus paternity analysis when related males compete for matings. Molecular Ecology, 6, 1155-1166.

Falconer DS (1981) Intoduction to Quantitative Genetics. 2nd ed. New York.

Frankel OH, Soule ME (1981) Conservation and Evolution. Cambridge University Press, Cambridge.

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. Genetical Research, Cambridge, 66, 95-107.

Frankham R (1998) Inbreeding and extinction: Island populations. Conservation Biology, 12, 665-675.

Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabek LM, Hoysak DJ (1990) Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. Science, 250, 1394-1397.

Haig SM, Ballou JD, Derrickson SR (1990) Management options for preserving genetic diversity: reintroduction of Guam rails to the wild. Conservation Biology, 4, 290-300.

Harris RB, Allendorf FW (1989) Genetically effective population size of large mammals: an assessment of estimators. Conservation Biology, 3, 181-191.

Hedrick PW, Miller PS (1992) Conservation genetics: techniques and fundamentals. Ecological Applications, 2, 30-46.

Hoelzel AR (1994) Genetics and ecology of whales and dolphins. Annual Review of Ecology and Systematics, 25, 377-399.

Hoelzel AR, Dahlheim M, Stern SJ (1998) Low genetic variation among killer whales (*Orcinus orca*) in the eastern North pacific and genetic differentiation between foraging specialists. Journal of Heredity, 89, 121-128.

Houlden BA, England PR, Taylor A, Greville WD, Sherwin WB (1996) Low genetic variability of the koala (*Phascolarctos cinereus*) in south eastern Australia following a severe population bottleneck. Molecular Ecology, 5, 269-282.

Hughes C (1998) Integrating molecular techniques with field methods in studies of social behavior: a revolution results. Ecology, 79, 383-399.

Keller LF, Arcese P, Smith JNM, Hochachka WM, Stearns SC (1994) Selection against inbred song sparrows during a natural population bottleneck. Nature, 372, 356-357.

Kenney RD, Hyman MAM, Owen RE, Scott GP, Winn HE (1986) Estimation of prey densities required by western North Atlantic right whales. Marine Mammal Science, 2, 1-13.

Knowlton AR, Kraus SD, Kenney RD (1992) Long-distance movements of North Atlantic right whales (*Eubalaena glacialis*). Marine Mammal Science, 8, 397-405.

Knowlton AR, Kraus SD, Kenney RD (1994) Reproduction in North Atlantic right

whales (*Eubalaena glacialis*). Canadian Journal of Zoology, 72, 1297-1305.

Kraus SD (1990) Rates and potential causes of mortality in North Atlantic right whales (*Eubalaena glacialis*). Marine Mammal Science, 6, 278-291.

Kraus SD (1991) Mating strategies in the North Atlantic right whale (*Eubalaena glacialis*). MSc. thesis, University of Massachusetts, Boston.

Kraus SD, Moore KE, Price CA, Crone MJ, Watkins WA, Winn HE, Prescott JH (1986) The use of photographs to identify individual North Atlantic right whale (*Eubalaena glacialis*). Report of the International Whaling Commission, Special Issue 10, 145-152.

Lacy RC (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology, 1, 143-158.

Lacy RC (1993) Impacts of inbreeding in natural and captive populations of vertebrates: Implications for conservation. Perspectives in Biology and Medicine, 36, 480-496.

Lacy RC, Alaks G, Walsh A (1996) Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. Evolution, 50, 2187-2200.

Lande R (1988) Genetics and demography in biological conservation. Science, 241, 1455-1460.

Larsen AH, Sigurjønsson J, Øien N, Vikingsson G, Palsbøll P (1996) Populations genetic analysis of nuclear and mitochondrial loci in skin biopsies collected from central and northeastern North Atlantic humpback whales (*Megaptera novaengliae*): population identity and migratory destinations. Proceedings of the Royal Society, London, 263, 1611-1618.

Lockyear C (1984) Review of baleen whale (Mysticeti) reproduction and implications for management. Report of the International Whaling Commission, Special Issue 6, 27-50.

Luikart G, Cornuet J (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology, 12, 228-237.

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology, 7, 963-974.

Lynch M, Conery J, Burger R (1996) Mutation accumulation and the extinction of small populations. American Naturalist, 146, 489-518.

Lyrholm T, Gyllensten U (1998) Global matrilineal population structure in sperm whales as indicated by mitochondrial DNA sequences. Proceedings of the Royal Society, London, 265, 1679-1684.

Malik S, Brown MW, Kraus SD, White BN (1999) Assessment of mitochondrial DNA structuring and nursery use in the North Atlantic right whale (*Eubalaena glacialis*). Canadian Journal of Zoology.

Malik S, Brown MW, Kraus SD, White BN (In Press) Analysis of mitochondrial DNA diversity within and between North and South Atlantic right whales. Marine Mammal Science.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology, 7, 639-655.

Milinkovitch MC, Meyer A, Powell JR (1994) Phylogeny of all major groups of cetaceans based on DNA sequences from three mitochondrial genes. Molecular Biology and Evolution, 11, 939-948.

Morin PA, Wallis J, Moore JJ, Chakraborty R, Woodruff DS (1993) Non-invasive sampling and DNA amplification for paternity exclusion, community structure, and

phylogeography in wild chimpanzees. Primates, 34, 347-356.

Nei M (1987) Molecular Evolutionary Genetics Columbia University Press, New York.

Nunney L, Elam DR (1994) Estimating the effective population size of conserved populations. Conservation Biology, 8, 175-184.

Oldfield ML (1988) Threatened mammals affected by human exploitation of the female-offspring bond. Conservation Biology, 2, 260-274.

Packer C, Pusey AE, Rowley H, Gilbert DA, Martenson J, O'Brien SJ (1991) Case study of a population bottleneck: lions of the Ngorongo Crater. Conservation Biology, 5, 219-230.

Paetkau D, Calvert W, Stirling I, Strobek C (1995) Microsatelite analysis of population structure in Canadian polar bears. Molecular Ecology, 4, 347-354.

Paetkau D, Waits LP Clarkson PL, Craigead L, Strobek C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. Genetics, 147, 1943-1957.

Palsbøll PJ, Allen J, Berubé M, Clapham PJ, Feddersen TP, Hammond P, Hudson RR,

Jørgensen H, Katona S, Larsen AH, Larsen F, Lien J, Mattila DK, Sigurjønsson J, Sears R, Smith T, Sponer R, Stevick PT, Øien N (1997) Genetic tagging of humpback whales. Nature, 388, 676-679.

Pemberton JM, Albon SD, Guinness FE, Clutton-Brock TH (1991) Countervailing selection in different fitness components in female red deer. Evolution, 45, 93-103.

Piper WH, Rabenold PP (1992) Use of fragment-sharing estimates from DNA fingerprinting to determine relatedness in a tropical wren. Molecular Ecology, 1, 69-78.

Payne R (1986) Long-term behavioural studies of the southern right whale (*Eubalaena australis*). Report of the International Whaling Commission, Special Issue 10, 161-167.

Payne R, Rowntree V, Perkins JS, Cooke JG, Lankester K (1990) Population size, trends, and reproductive parameters of right whale (*Eubalaena australis*) off Peninsula Valdes, Argentina. Report of the International Whaling Commission, special issue, 12, 271-278.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution, 43, 258-275.

Ralls K, Brugger K, Ballou JD (1979) Inbreeding and juvenile mortality in small populations of ungulates. Science, 206,1101-1103.

Ralls K, Ballou JD, Templeton AR (1988) Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology, 2, 185-193.

Reeves RR, Mitchell ED (1986a) The Long Island, New York right whale fishery:1650-1924. Report of the International Whaling Commission, Special Issue 10, 201-220.

Reeves RR, Mitchell ED (1986b) American pelagic whaling for right whales in the North Atlantic. Report of the International Whaling Commission, Special Issue 10, 221-245.

Rice DW (1998) Marine mammals of the world: systematics and distribution. The Society for Marine Mammalogy, Special Publication 4, 231 p.

Ritland K (1996) Estimators for pairwise relatedness and individual inbreeding coefficients. Genetic Research, 67, 175-185.

Rockwell RF, Barrowclough GF (1995) Effective population size and lifetime reproductive success. Conservation Biology, 9, 1225-1233.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly population. Nature, 392, 491-494.

Schaeff CM, Kraus SD, Brown MW, White BN (1993) Assessment of the population

structure of western North Atlantic right whales (*Eubalaena glacialis*) based on sighting and mtDNA data. Canadian Journal of Zoology, 71, 339-345.

Schaeff CM, Kraus SD, Brown MW, Perkins JS, Payne R, White BN (1997) Comparison of North and South Atlantic right whale (*Eubalaena*) genetic variability using DNA fingerprinting. Canadian Journal of Zoology, 75, 1073-1080.

Schevill WE (1986) Right whale nomenclature. Report of the International Whaling Commission, Special Issue 13, 217-223.

Schevill WE, Watkins WA, Moore KE (1986) Status of *Eubalaena glacialis* off Cape Cod. Report of the International Whaling Commission, Special Issue 10, 79-82.

Soule ME (1986) Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, Massachusetts.

Soule ME (1987) Viable populations for conservation. Cambridge University Press, Cambridge.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research, 17, 6463-6471.

Taylor AC, Sherwin WB, Wayne RK (1994) Genetic variation at microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat (*Lasiorhinus krefftii*). Molecular Ecology, 3, 277-290.

Thornhill NW (1993) The Natural History of Inbreeding and Outbreeding. Chicago University Press, Chicago.

Valsecchi E, Amos W (1996) Microsatellite markers for the study of cetacean populations. Molecular Ecology, 5, 151-156.

Vucetich JA, Waite TA, Nunney L (1997) Fluctuating population size and the ratio of effective to census population size. Evolution, 51, 2017-2021.

Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics, 121, 379-391.

Waples RS (1991) Genetic methods for estimating the effective size of cetacean populations. Report of the International Whaling Commission, Special Issue 13, 279-301

.

Waser PM, Strobek C (1998) Genetic signitures of interpopulation dispersal. Trends in Ecology and Evolution, 13, 43-44.

Watkins WA, Schevill WE (1982) Observations of right whales, *Eubalaena glacialis*, in Cape Cod waters. Fishery Bulletin, 875-880.

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American Journal of Human Genetics, 44, 388-396.

Wildt DE, Bush M, Goodrowe KL, Packer C, Pusey AE, Brown JL, Joslin P, O'Brien SJ (1987) Reproductive and genetic consequences of founding isolated lion populations. Nature, 329, 328-331.

Winn HE, Price CA, Sorensen PW (1986) The distributional biology of the right whale (*Eubalaena glacialis*) in the western North Atlantic. Report of the International Whaling Commission, Special Issue 10, 129-138.

Wright S (1931) Evolution in Mendelian Populations. Genetics, 16, 97-159.

Chapter Two

Characterisation and Isolation of microsatellite loci from the endangered North Atlantic right whale

As a consequence of historic whaling pressures, the western North Atlantic right whale (*Eubalaena glacialis*) is one of the most endangered of all large cetaceans (Knowlton *et al.* 1994). Photo-identification techniques for individual identification of whales and over 19 years of monitoring have provided extensive behavioural and life history data on most of the known population (Kraus 1990; Knowlton *et al.* 1994). However, some whales have not been identified due to low quality photographs. The development of individual-specific genetic profiles would augment the study by allowing these whales to be identified (see Palsbøll *et al.* 1997).

Microsatellite loci are known to have high levels of genetic variability in many taxa due to the high rates of mutation (Dietrich *et al.* 1992; Bruford and Wayne 1993). Moreover, as microsatellites are non-coding, allele frequencies will be subject to random genetic drift, making these markers useful for studying levels of genetic diversity and population structuring (Nauta and Weissing 1996).

Skin samples were collected from *E. glacialis* in the waters off eastern Canada between 1988 and 1997. Samples were stored in DMSO (20% DMSO, 0.25 M sodium-EDTA, saturated with NaCl, pH 7.5; Amos and Hoelzel 1991) and DNA was extracted using phenol-chloroform extractions (Brown *et al.* 1991; Schaeff *et al.* 1993). Samples were also available for several South Atlantic right whales (*E. australis*) from near Peninsula Valdes, Argentina (Schaeff *et al.* 1993).

Restriction digests of 1-3 μg of genomic DNA were conducted in 25 uL volumes using three blunt-end cutting restriction enzymes (*Rsa*1, *Eco*RV, and *Alu*1; Gibco-BrL). DNA fragments of < 450 base pairs were size separated on a 2% agarose gel. Cloning

was carried out as described in Rassman *et al.* (1991). Insert sequences were determined using the PRISM™ Ready Reaction Dye Deoxy Termination kit (Applied Biosystems Inc.), a Perkin-Elmer 9600 Thermal Cycler, and an Automated DNA Sequencing System (373A, Applied Biosystems). Primers were developed for the 13 of 34 microsatellite sequences identified (Primer v. 5; Lincoln *et al.* 1991).

Amplifications using the Polymerase Chain Reaction (PCR) used 20-50 ng of DNA, 0.2 uM of each primer, 20 nm of γ-^{33}P end-labelled primer, 2.0-2.5 mM $MgCl_2$, 0.70 U of *Taq*, 1x reaction buffer, and 0.20 mM dNTPs (Gibco-BrL). PCR products were size separated on a 6% denaturing, polyacrylamide gel and visualised by autoradiography. Alleles were sized relative to the clones. Loci were tested for deviations from Hardy-Weinberg equilibrium (HWE) when $\geq$ 58 chromosomes had been screened (Exact test; Raymond and Rousset 1995).

Loci were considered monomorphic when the most common allele had a frequency of > 95% (N $\geq$ 58 chromosomes). Only two of 13 and one of nine polymorphic loci deviated significantly from HWE in *E. australis* and *E. glacialis* (rw26 and rw18 and rw31 and rw34, respectively). The number of alleles (allelic diversity) was as great or greater in *E. australis* than in *E. glacialis* at all but a single locus (rw34).

The potential use of these microsatellites in the study of toothed whales was assessed using seven randomly selected primer pairs on six beluga whale samples (*Delphineptera leucas*). All seven primer pairs produced PCR products and despite the small sample size, four of the seven loci were variable (rw2-17, rw2-19, rw21, and rw26).

For the right whales, the microsatellite loci will allow individual-specific profiles to be developed and for *E. glacialis*, the exclusionary power (PE) for paternity analysis is over 97% when the identity of one parent is known. In contrast, high allelic diversity in *E. australis* provides a PE ≅ 99% when neither parent is known. These microsatellite-based profiles will augment the *E. glacialis* catalogue by identifying new whales and confirming the identity of whales when photographs are of low quality.

Table 2-1. Microsatellite loci identified from the genome of the North Atlantic right whale.

Locus	Repeat sequence Genbank Accession Number	Primer sequence (5' > 3') FORWARD	PCR product (bp)	T_a (°C)[5]	No. Alleles		H^1		Nc^2	
					nA*	sA**	nA	sA	nA	sA
rw18	$(TG)TA(TG)_{19}$ AF156294	AGAGGGAAGCAAACTGGA GAAGGNTGCCAGACACCC†	195	52	5	16	0.5	1	378	58
rw26	$(TG)_{16}(TA)_2$ AF156295	GTCCATCCATATTACTGC CAGTTATACCTCAATGAAGC	165	56	2	11	0.4	1	386	54
rw31	$(TG)_{20}$ AF156296	TATTCATGGAGTGCTTTGG CCTAGAGTCCAGTGTGGTA	130	54	5	7	0.6	0.8	354	50

rw25	$(TG)_{18}$ AF156556	CTTAACATGGAAGGCTCCC GCCAAGCATTGGGACTTTTG	140	54	1	4	-	-	60	20
rw2-17	$(GT)_5A(GT)_{13}$ AF156297	ATCTGGCATTTGTTTTAAAATAATCC CCAGAAAGAATAATGTAATAAACCC	166	52	1	3	-	0.4	60	58
rw2-19	$(AC)_{12}$ AF156298	AGTTCCATAGGGCTGCTCAC TTCCATTTTTGGGTTCAATC	96	52	1	5	-	0.7	60	58
rw4-10	$(GT)_{17}$ AF156555	ATGGCATTACTTCATTCTTT GCCAAACTTACCAAATTGTG	177	54	2	9	0.3	0.8	50	26
rw34	$(CA)_{25}$ AF156299	AGCCCCATAACGGCGCATA GGGAGCCAGAACCTGATAC	122	57	11	2	0.8	-	368	18
rw48	$(TG)_{23}$ AF156300	CCAATGACTTTTCCCTGTA GATACCGCAGTGTGTCCTG	112	57	6	6	0.4	-	370	22

rw2-12	$(TG)_4A(TG)_2ACGCACAC(GT)_7T(TG)_5$ AF156301	TGACACTTTTCCGCTTTAGG AAAAGCTTCCATCCTAACCA	86	52	1	2	-	-	60	20
sam25	$(TG)_{16}(TA)_2$ AF156302	CTGCAAATGGCATTACTTC CCAAACTTACCAAATTGTG	182	53	2	7	-	-	20	18
rw4-5	$(TG)_{15}TATGTAT(GA)_{10}AT(GT)_2$ AF156303	AGGTCTTTCATTGCTGCC ACGGAAATCAGAAAGCCTTA	115	55	2	6	-	-	18	20
rw4-17	$(TG)_{18}A(T)_8$ AF156304	TATCCTGCAACCTTGCTGA TCACAGATGACATGACCTTG	104‡	55	4	9	0.7	-	362	22

[1]Observed heterozygosity, [2] Chromosomes screened; [3]North Atlantic right whale; [4]South Atlantic right whale; [5]annealing temperature. PCR cycles consisted of 5min. at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 52-57°C, 45 s at 72°C and a final 10 min step at 72°C. [6]Heterozygote deficiency ($p \leq 0.01$). [7]Heterozygote excess ($p < 0.05$). † Sixth position was represented by both G and C. ‡ In addition to the ~104 bp target product, rw4-17 had a 'fixed' band of 80 bp in both species. No size overlap was observed between alleles from the target region and the 'fixed' band for either species.

Acknowledgements

This research was supported by the NMFS (U.S.A.), WWF (Canada), and grant and scholarship support from NSERC Canada (to BNW and RCW, respectively).

Literature cited

Amos W, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. . Report of the International Whaling Commission, Special Issue 13, 99-104.

Brown MW, Kraus SD, Gaskin DE (1991) Reaction of North Atlantic right whales (*Eubalaena glacialis*) to skin biopsy sampling for genetic and pollutant analysis. Report of the International Whaling Commission, Special Issue 13, 81-90.

Bruford MW, Wayne RK (1993) Microsatellites and their application to population genetics. Current Opinion in Genetics and Development, 3, 939-943.

Dietrich W, Katz H, Linclon SE (1992) A genetic map of the mouse suitable for typing intraspecific crosses. Genetics, 131, 423-447.

Knowlton AR, Kraus SD, Kenney RD (1994) Reproduction in North Atlantic right whales (*Eubalaena glacialis*). Canadian Journal of Zoology, 72, 1297-1305.

Lincoln SE, Daly JJ, Lander ES (1991) Primer: a computer program for automatically selecting PCR primers. Version 0.5.

Nauta MJ, Weissing FJ (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics, 143, 1021-1032.

Palsbøll PJ, Allen J, Berubé M, Clapham PJ, Feddersen TP, Hammond P, Hudson RR, Jørgensen H, Katona S, Larsen AH, Larsen F, Lien J, Mattila DK, Sigurjønsson J, Sears R, Smith T, Sponer R, Stevick PT, Øien N (1997) Genetic tagging of humpback whales. Nature, 388, 676-679.

Rassman K, Schlotterer C, Tautz D (1991) Isolation of simple-sequence loci for use in polymerse chain reaction-based DNA fingerprinting. Electrophoresis, 12, 113-118.

Raymond M, Rousset F (1995) Genepop (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86, 248-249.

Schaeff CM, Kraus SD, Brown MW, White BN (1993) Assessment of the population structure of western North Atlantic right whales (*Eubalaena glacialis*) based on sighting and mitochondrial DNA RFLP data. Canadian Journal of Zoology, 71, 339-345.

Chapter three

Evaluating the status of an endangered species using genetic identities: the North Atlantic right whale.

Abstract

Monitoring the highly endangered North Atlantic right whale since 1980 has led to the photo-identification of 388 individuals of which 283 were living.[1] Over 17 years of monitoring have also resulted in the collection of individual-specific survivorship and reproductive data. Using these data, a population model has been constructed that predicts the extinction of this species within the next 200 years.[2] To evaluate the status of the population, individual-specific genetic profiles were developed for 209 whales at nine microsatellite loci and the mitochondrial DNA control region.[3,4] Using these profiles, we were able to assess the current estimate of the census population size and provide the first effective population size estimate for this species. Apparent mating behaviour suggested that mating involved dominance polygyny[5], however, genetic paternity analysis identified that 83% of the calves had different fathers (20/24). This finding is consistent with a random mating strategy and places the effective population size at *ca.* 133. Although mating appears to be random, population structuring was identified between calves born to two groups of females that differ in their use of nursery habitat ($p < 0.001$). Reproductive isolation is incomplete, however, and the migration rate between the two groups is *ca.* one individual/generation. Genetic analyses of the North Atlantic right whale, particularly the identification of new adults, suggest that the population size is larger than the 1997 estimate of 283.

Introduction/Discussion

The North Atlantic right whale is one of the most critically endangered of all large cetaceans.[6] A 17-year study by the North Atlantic Right Whale Consortium, a group consisting of American and Canadian researchers, has resulted in the compilation of a photographic catalogue of 388 individual animals,[1] 283 of which were alive in 1997. Using these data, Caswell *et al.*[2] have modelled the growth rate for the population. This model predicts the extinction of this species within 200 years due to the combined effects of low individual survivorship and the small number of calves born each year. All of these estimates and models rely, however, on the effectiveness of the existing monitoring program at identifying newborns and matching sightings to previously identified animals.

Although most births since 1980 are thought to have been recorded off the coasts of Florida and Georgia, a few new juveniles and full grown animals are sighted and added to the catalogue each year.[7] Although the reproductive females in this population appear to share one common calving area,[9] differences exist in where they take their calves to nurse.[4, 10] Since 1980, an average of 11 calves have been born each year, and only 60% of these (123/205) were taken to the nursery ground in the Bay of Fundy (BoF).[4, 10] As the location of the alternate nursery area is unknown, those calves that are not taken to the Bay of Fundy by their mothers are not photoidentified. The phenotypic traits for visual identification only begin to appear around the time when mother-calf pairs have left the calving ground for the feeding and nursery habitat areas in the north.[8] As a result, these calves will appear as 'new sightings' in later years. It is unknown whether whales that are sighted for the 'first time' after their year of birth represent known births for which

photo-identifications were not made or previously unknown whales. Between 1980 and 1997, for example, 35 calves were not identified in their year of birth and 19 new sightings were recorded. If most new sightings represent new animals, the population may be larger than the present estimate. However, as the mating system appears to involve dominance polygyny, the census population size may not reflect the effective number of breeders.

To test the hypothesis that new sightings represent those calves that were not identified during their first year, and assess the current population size estimate, we attempted to assign mothers to 18 new sightings. Maternity analysis was conducted using two groups of putative mothers: those mothers known to have unidentified calves and those adult females that have not been observed with a calf. The proportion of breeding males in the population was then estimated through paternity analysis and used to estimate the effective population size (N_e).[12]

Maternity assignments were made using individual-specific genetic profiles based on nine microsatellite loci and mtDNA control region haplotype sequences.[3,4] Maternity assignments were made if all nine microsatellite loci and the mitochondrial haplotypes matched between a new sighting and an adult female. Collectively, 42 adult females were available for maternity testing to the 18 new sightings. In all but two instances, all of the females could be excluded as mothers. Both of the two maternity matches were assigned to unidentified calves born during the 1998 calving season. One of the matched calves, which had been found dead in the calving ground (Tmp18, sampled 11 Jan. '98), was matched to a female whose last known calving event was in 1991 (New England

Aquarium identification 1158). Female 1158 was also not one of the seven mothers identified as having had a calf in 1998.[1] The second calf (Tmp20, sampled 30 Jan. '98) had been biopsy-sampled while in the presence of a female (NEAq 1315), however, the relationship between these two whales was uncertain. Maternity analysis using all putative mothers verified that calf Tmp20 was the offspring of female 1315.

All of the remaining 'new' sightings were either full grown or subadult when first encountered, suggesting that they may represent 16 of the 32 unidentified calves born between 1980 and 1995.[1] Given the incomplete sampling of new sightings and mothers available for the maternity analysis, four maternal matches would be expected, on average (assuming a hypergeometric distribution).[13] No matches were made between the eight mothers with unidentified calves that were available for maternity testing. Moreover, no matches were made between the 33 females that were not known to have produced calves regularly during the 18-years of monitoring and the new sightings. The failure to match even a single new sighting to one of the 42 females, assuming they represent unidentified calves, is unlikely ($p < 0.0001$, exact test assuming a hypergeometric distribution). These results suggest that many of these new sightings are previously unknown adults or subadults. These 16 unknown whales may, therefore, represent a subset of the population that are infrequent visitors to the areas that are routinely monitored along the east coast of North America.

The North Atlantic right whale population is known to consist of two groups of breeding females that differ in their use of habitat areas.[4,9] MtDNA structuring between these two groups indicates that one group consists of mother-calf pairs that feed and nurse

in the Bay of Fundy (BoF) during the summer and that the second consists of mother-calf pairs that use some alternate summer habitat (Non-BoF). The existence of at least one unidentified habitat area is consistent with the finding that some new sightings represent adults that have not been observed within the areas typically monitored by the Right Whale Consortium group.

In order to identify whales with unknown birthing histories, including new sightings, as members of the BoF or Non-BoF groups, the population was examined for evidence of further genetic structuring. All of the genotyped calves born between 1980 and 1997 were divided into two groups: those taken to the Bay of Fundy (BoF, N = 54) and those that were taken elsewhere (Non-BoF, N = 24). Significant genetic structuring was observed between the two groups ($R_{st} = 0.19$, $p < 0.001$)[14]. In order to discriminate between BoF and Non-BoF whales, allele frequencies from each of the two groups were used to identify the likelihood of individual whales belonging to one or other of the two groups. For each individual genotype, the log-likelihood of an observed allele originating from each of the two populations was calculated based on the frequency of that allele in each population (Table 3-1).[15] The LOG likelihood ratios for each allele were then summed (p_I) and used as the index of similarity (*i.e.*, Individual index: I_i) to each of the two populations (BoF and Non-BoF; $\{I_i = \Sigma\log(p_{iBoF}/p_{iNBoF})\}$). The key difference in the two distributions of I_i values for the calves is the presence of a second peak in the distribution within the BoF group (Figure 3-1). This second peak consists of 24 of the 54 Bay of Fundy calves, all of which have positive I_i values falling outside the range of the main BoF distribution (*i.e.*, $I_i > 2.25$). In order to identify unifying characteristics of the BoF

extreme calves, sighting and birth histories were considered for each of the 24 calves with respect to their pedigrees (Figure 3-2). The 24 calves were found to represent 12 family groups, some of which included individuals from two or three different generations.

When the I_i values are considered for all whales with unknown nursing histories (N = 111), the distribution is non-normal (Kolmogorov-Smirnoff test $p < 0.001$) and has a separate group of whales with large I_i values > 2.25 (Figure 3-3). The group of unknown whales with I_i values greater than 2.25 included seven of the 11 sampled mothers of the unique BoF calves, one adult female with no genotyped calves, and 11 adult males. All but one of these mothers also had I_i values that fell well outside the distribution of Non-BoF whales (*i.e.*, $I_i > 1.0$). Although the birth history is unknown for the 11 males identified as members of this group, five had a mtDNA haplotype (haplotype C) that is only present in two of the 11 mothers identified as belonging to this group. This finding provides further evidence that this unique Bay of Fundy group includes additional, unsampled adult females.

The calves belonging to the Non-BoF and the BoF group with $I_i > 2.25$ ('BoF extremes') may represent the progeny of two discrete breeding populations that were once reproductively isolated but are now exchanging genes. Under this hypothesis, the population would consist of three different groups, the Non-BoF population, the BoF extreme population, and a large group consisting of progeny of crossings between the Non-BoF and BoF extreme groups.

The estimated immigration rate between the BoF extreme and Non-BoF populations is one individual per generation (Rho estimate; N = 100).[14] As such, we tested the

observed distribution of I_i values for calves of BoF extreme mothers to those expected if these females mate randomly with males in the population. Matings were simulated between the 10 BoF extreme mothers and 100 randomly selected adult males from the overall population. Only 18.8% of the simulated matings produced calves with I_i values > 2.25 (Figure 3-4). Based on this distribution, only 10 of the 55 calves that have been produced by these 10 mothers would be expected to have I_i values above 2.25. The observed distribution of I_i values differed from the simulated matings (χ^2; $p < 0.001$). Of the 55 births to the BoF extreme families, 24 calves had an I_i value > 2.25. The large number of calves with higher I_i values than were predicted from simulated random matings ($p < 0.001$, χ^2) supports the hypothesis that BoF extreme mothers are not mating randomly with adult males in the overall population. In fact, even if all 22 of the remaining, unsampled calves had I_i values < 2.25, the observed proportion of calves with I_i > 2.25 within these families would still be substantially larger than the predicted level (70% of sampled calves versus 42% of all calves).

Analysis of 18-years of sighting-resighting data has identified other differences in calf production among adult females in this population.[1] Females are capable of producing calves at four year intervals;[11] as such, a population with 100 adult females could produce an average of 25 calves per year. Although an estimated 100 adult females are present in the population in any given year, the population has only averaged 11 births per year from 1980 to 1997.[11] The difference between the potential and realized number of calves is the result of some females never having produced a calf during this time, and the failure of many of the successful breeders to produce more than one calf. In the 1997

population, for example, only 73 females had ever produced calves, and 55% of these had only produced a single calf during their lifetime.[16] The reasons for the low calf production remain speculative,[17-19] however, survivorship and reproductive fitness may differ between BoF and Non-BoF females due to differences in the quality of their respective habitats.[1,16] Although 60% of all calves are taken to the BoF,[4,10] in 1997 and 1998, only nine of the 24 calves produced were born to BoF females.[1] Until the location of the alternate nursery ground and the mating ground(s) are known, efforts to mitigate any existing disturbances within these habitats will not be possible.

Neither the timing nor the location of mating activities are known for this species, however, behavioural data suggests that males compete for access to females.[4] Such a mating system would reduce the N_e and increase the incidence of breeding among individuals with alleles that are identical by descent.[20] N_e may even be smaller given the reproductive isolation between BoF and Non-BoF populations. Using DNA samples from 89 adult males (73 males over five years old and 16 of unknown age) paternity analysis was conducted using exclusion and LOD likelihood analyses in combination with sighting records.[15] LOD values represent the probability of a male being the true father relative to a random male from the population. Therefore, LOD values are useful for ranking non-excluded males based on likelihood estimates. Using this approach, 20 different fathers were assigned to 24 calves with > 80% match probability based on the differences in LOD scores.[16] No evidence of inbreeding was observed among mating pairs. In fact, the relatedness coefficients between members of mating pairs were

Table 3-1. Allele frequencies at microsatellite loci for North Atlantic right whale subpopulations.

Allele (bp)	Non-BoF	BoF* calves	BoF extreme	All BoF extreme
1P				
119		0.01		
132	0.02			
135	0.58	0.67	0.57	0.64
137	0.40	0.32	0.43	0.36
37M				
199	0.48	0.36	0.45	0.40
201	0.46	0.57	0.55	0.56
203	0.06	0.07		0.04
Rw18				
191	0.11	0.03	0.05	0.03
193	0.02	0.01		
195	0.56	0.71	0.74	0.71
197	0.25	0.21	0.19	0.22
201	0.06	0.04	0.02	0.04
Rw26				
163	0.33	0.24	0.24	0.28
165	0.67	0.76	0.76	0.71
167				0.01

Allele (bp)	Non-BoF	BoF calves	BoF extreme	All BoF extreme
IgF				
151		0.01	0.09	0.12
153	0.04	0.02		
155	0.17	0.05		
157	0.08	0.02	0.05	0.04
159	0.38	0.44	0.45	0.41
161	0.17	0.11	0.19	0.18
163	0.04	0.03		0.01
165	0.10	0.26	0.19	0.22
167		0.01		
169	0.02	0.05	0.03	0.02
Rw31				
122	0.27	0.16	0.07	0.11
128	0.63	0.01	0.16	0.19
130		0.75	0.67	0.62
132	0.06	0.07	0.10	0.07
136	0.04	0.01		0.01

Allele (bp)	Non-BoF	BoF calves	BoF extreme	All BoF extreme
Rw34				
100	0.54	0.49	0.62	0.56
108	0.02	0.06	0.02	0.03
110	0.02	0.03	0.02	0.01
114	0.19	0.08	0.05	0.06
116	0.13	0.21	0.17	0.19
118	0.04	0.04		0.01
120	0.02	0.04	0.07	0.08
122	0.02	0.04		0.04
124	0.02	0.01	0.05	0.02
Rw48				
104		0.01		0.02
110	0.04	0.07	0.10	0.10
112	0.19	0.12	0.07	0.05
114	0.77	0.79	0.57	0.60
116		0.01	0.26	0.23
Rw410				
104	0.48	0.47	0.34	0.46
106	0.32	0.30	0.28	0.23
108	0.03	0.04	0.02	0.04
110	0.17	0.19	0.36	0.27

*BoF allele frequencies excluding BoF extreme calves where the latter refers to individuals found to have an I_i values > 2.25.

Figure 3-1. Plots of Individual Index values (I_i) for North Atlantic right whales from different nursery groups. The I_i is the log-likelihood of an individual's genotype being derived from each of two putative populations (Bay of Fundy and Non-Bay of Fundy), where $I_i = \Sigma\log(p_{ij}/p_{ik})$, where p is the frequency of allele 'i' in the Bay of Fundy group (j) and in the Non-Bay of Fundy group (k). The main distribution of I_i values fall around 0 for both groups and has a large area of overlap at moderate I_i values. A second peak of more positive I_i values (Ii > 3.0) exists in the Bay of Fundy group.

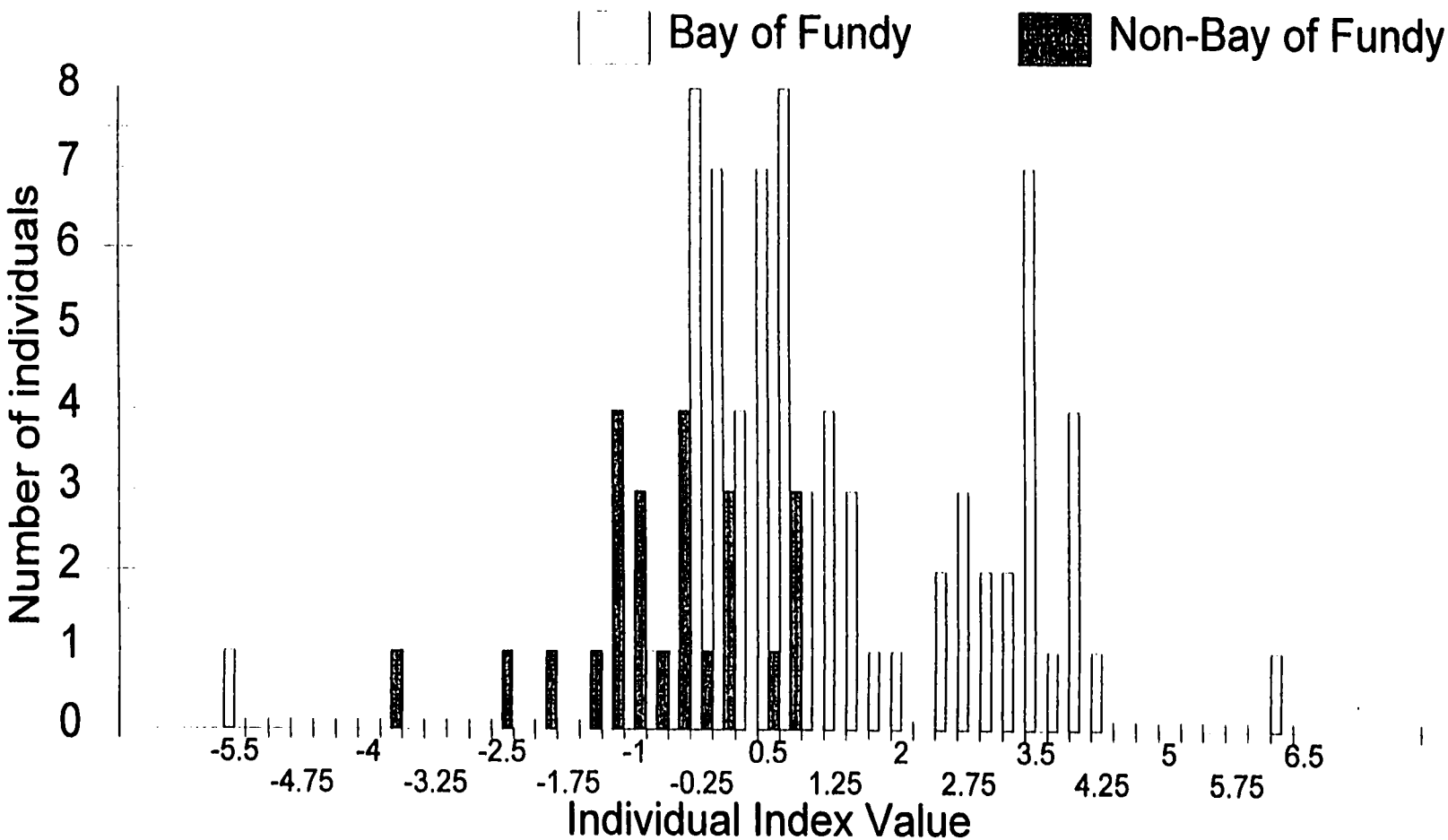

Bay of Fundy
Non-Bay of Fundy
Number of individuals
0
1
2
3
4
5
6
7
8
-5.5
-4.75
-4
-3.25
-2.5
-1.75
-1
-0.25
0.5
1.25
2
2.75
3.5
4.25
5
5.75
6.5
Individual Index Value

Figure 3-2. Individual index values (I_i) and maternally-based pedigrees for Bay of Fundy extreme calves. I_i values consistent with the Bay of Fundy subgroup are indicated in bold ($I_i > 2.25$). The 24 calves represent 12 separate maternal lineages that include one or two generations of calves. Four-digit numbers associated with each whale represent the individual New England Aquarium identification code assigned based on photo-identification records. Unidentified whales and non-sampled whales are indicated by question marks. The four mitochondrial haplotypes observed in the family groups are shown for the oldest known female in each pedigree (haplotypes A, B, C, and D).

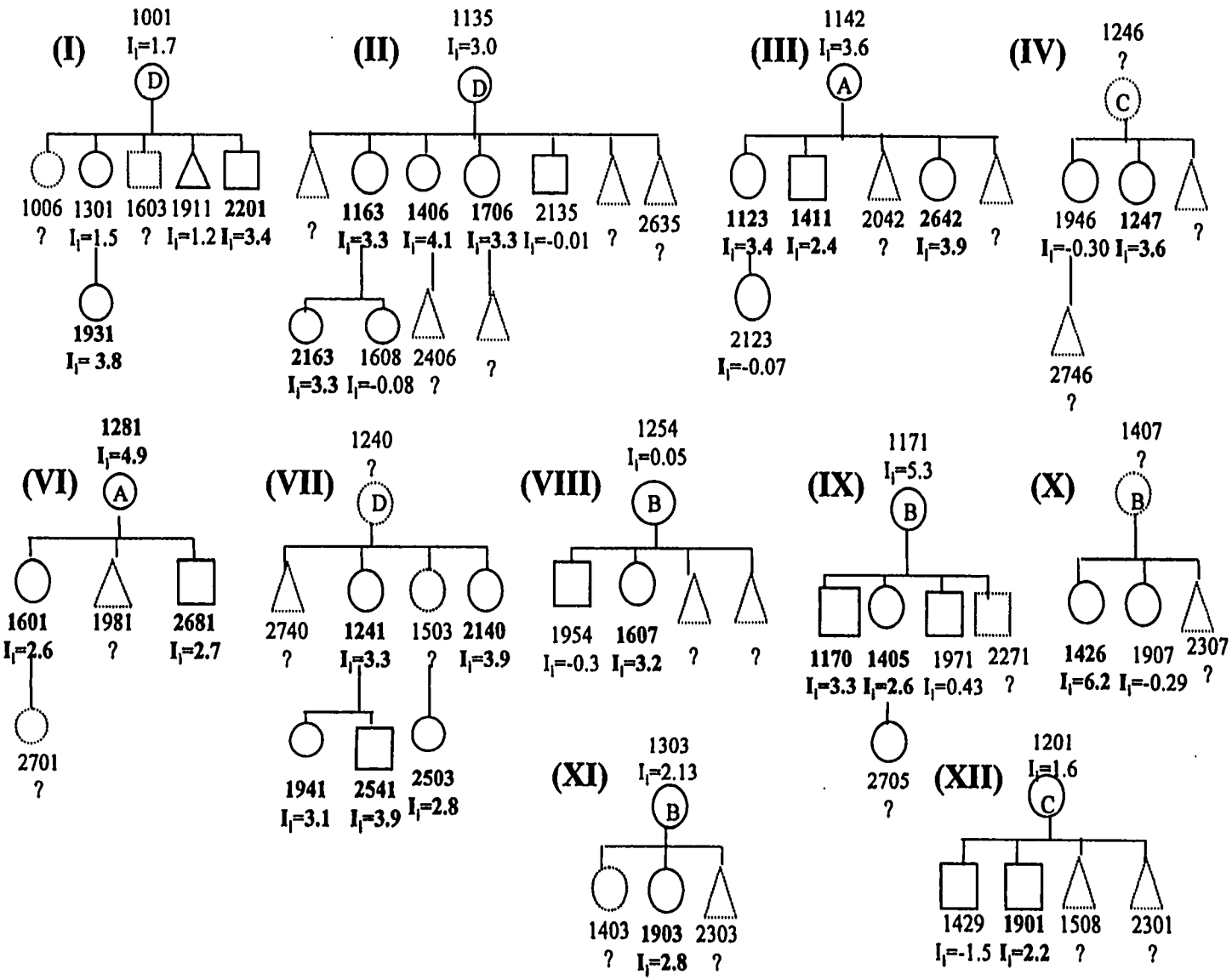

(I)
1001
I_i=1.7
D
1006 1301 1603 1911 2201
? I_i=1.5 ? I_i=1.2 I_i=3.4
1931
I_i= 3.8
(II)
1135
I_i=3.0
D
? 1163 1406 1706 2135 2635
I_i=3.3 I_i=4.1 I_i=3.3 I_i=-0.01 ? ?
2163 1608 2406 ?
I_i=3.3 I_i=-0.08 ?
(III)
1142
I_i=3.6
A
1123 1411 2042 2642 ?
I_i=3.4 I_i=2.4 ? I_i=3.9
2123
I_i=-0.07
(IV)
1246
?
C
1946 1247 ?
I_i=-0.30 I_i=3.6
2746
?
(VI)
1281
I_i=4.9
A
1601 1981 2681
I_i=2.6 ? I_i=2.7
2701
?
(VII)
1240
?
D
2740 1241 1503 2140
? I_i=3.3 ? I_i=3.9
1941 2541 2503
I_i=3.1 I_i=3.9 I_i=2.8
(VIII)
1254
I_i=0.05
B
1954 1607 ? ?
I_i=-0.3 I_i=3.2
(IX)
1171
I_i=5.3
B
1170 1405 1971 2271
I_i=3.3 I_i=2.6 I_i=0.43 ?
2705
?
(X)
1407
?
B
1426 1907 2307
I_i=6.2 I_i=-0.29 ?
(XI)
1303
I_i=2.13
B
1403 1903 2303
? I_i=2.8 ?
(XII)
1201
I_i=1.6
C
1429 1901 1508 2301
I_i=-1.5 I_i=2.2 ? ?

Figure 3-3. Plots of Individual Index (I_i) values for the North Atlantic right whale population. The observed distribution is significantly different from a normal distribution ($p < 0.001$).

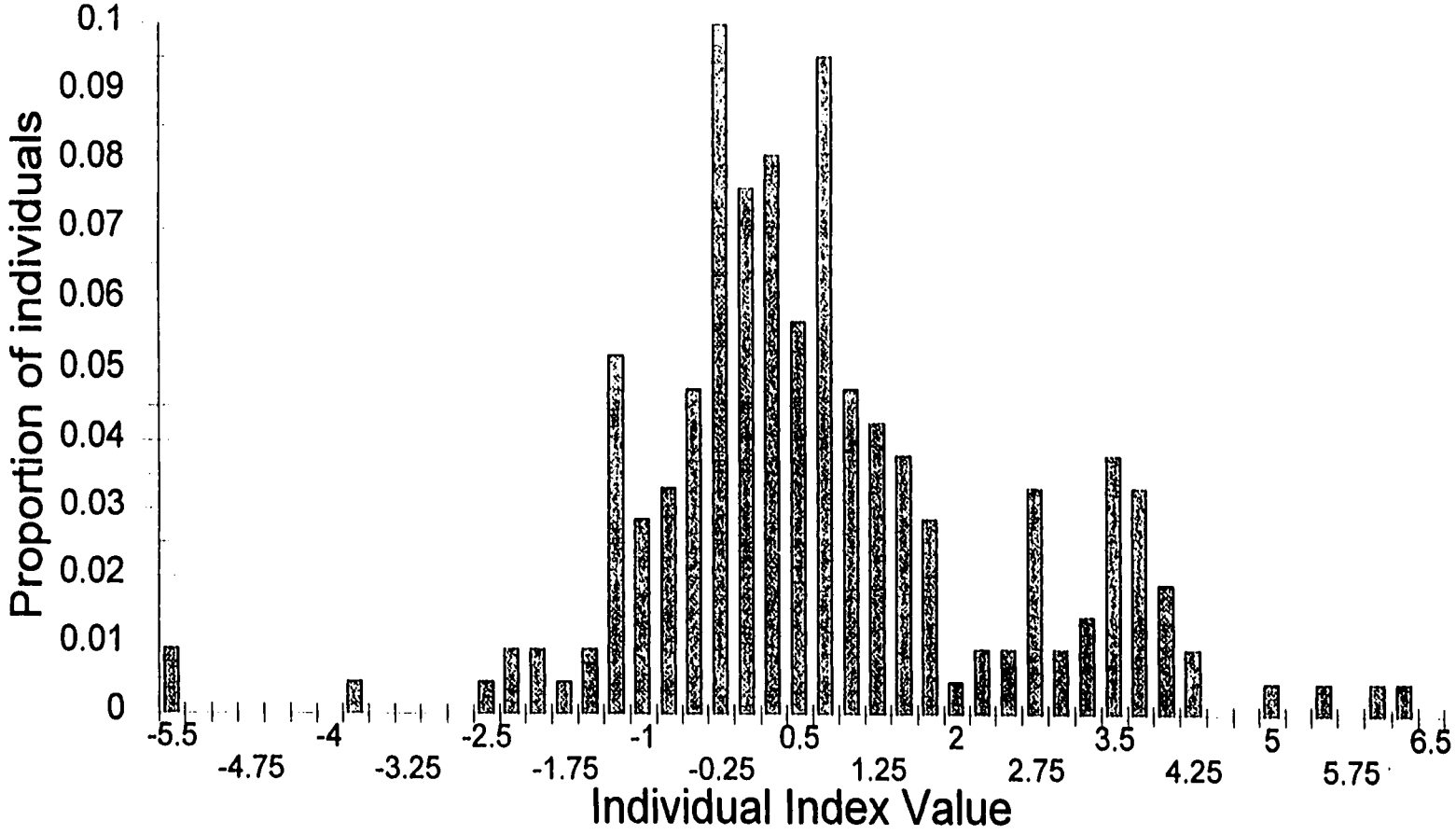
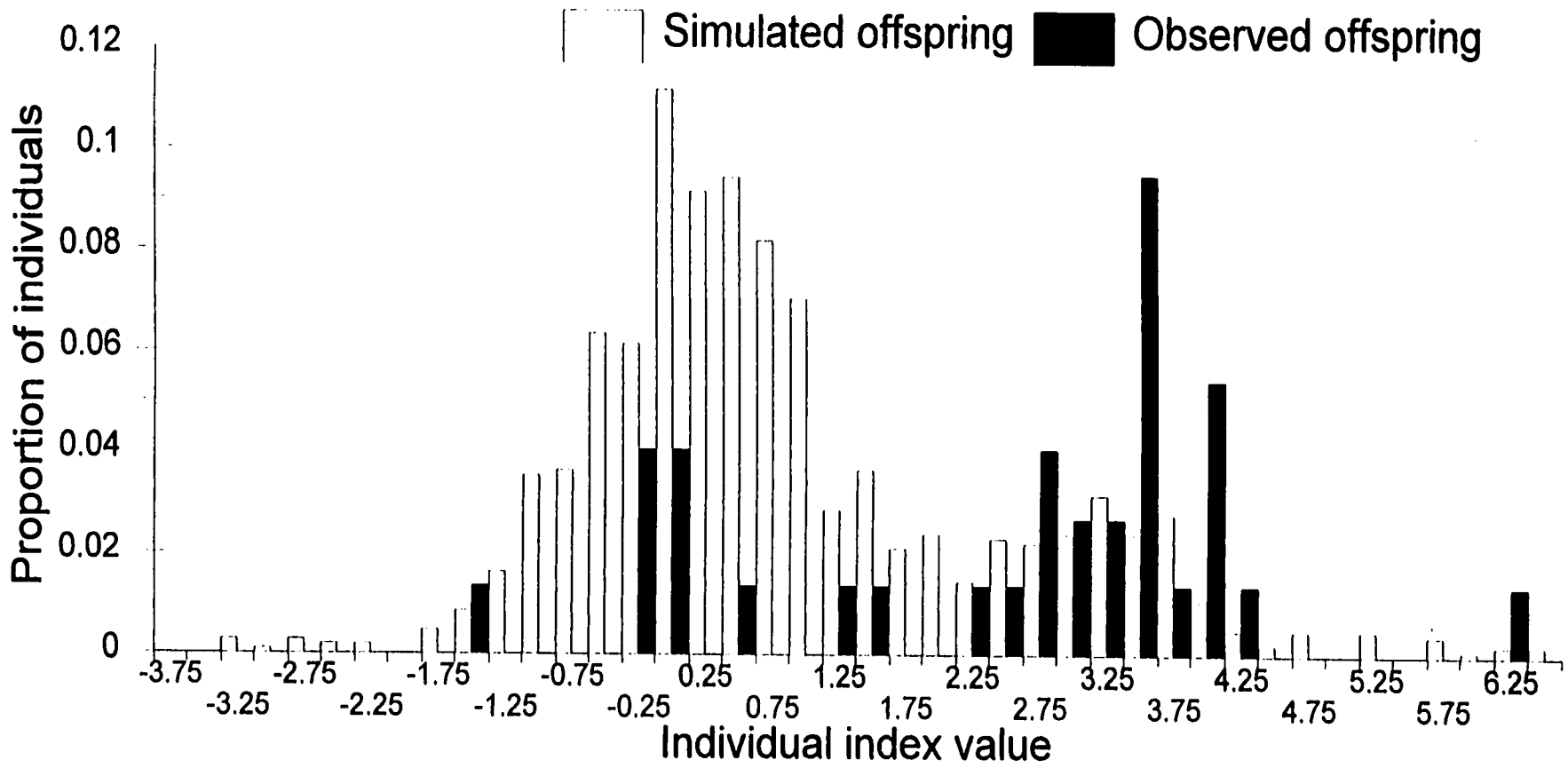

Proportion of individuals
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
-5.5
-4.75
-4
-3.25
-2.5
-1.75
-1
-0.25
0.5
1.25
2
2.75
3.5
4.25
5
5.75
6.5
Individual Index Value

Figure 3-4. Individual Index (I_i) values for simulated offspring of BoF extreme mothers under random mating. I_i values from simulated random matings between males from the general population (*i.e.*, all males pooled) and BoF extreme mothers are shown relative to the observed I_i values for 33 of the 55 calves born to these females (*i.e.*, those that were genotyped).


Simulated offspring
Observed offspring
Proportion of individuals
0.12
0.1
0.08
0.06
0.04
0.02
0
-3.75
-3.25
-2.75
-2.25
-1.75
-1.25
-0.75
-0.25
0.25
0.75
1.25
1.75
2.25
2.75
3.25
3.75
4.25
4.75
5.25
5.75
6.25
Individual index value

consistent with values expected among unrelated animals (R_{xy} = -0.035 $\pm$ 0.28 versus 0.0007 $\pm$ 0.24, respectively).[16]

Given that 83% of the calves were assigned different fathers, the effective population size (N_e) was estimated by assuming that the proportion of fathers would remain similar for the entire population. In the living population in 1997, 73 of the 101 adult females produced calves.[16] Many of these females only produced a single calf during the 17-year period examined. If each of the 73 females had only produced a single calf, 60 different fathers would be expected to sire the resulting 73 offspring. The N_e would therefore be 133. If, on the other hand, mating involved extreme harem polygyny and only 20% of the males bred successfully, the N_e would be less than half of the first estimate (*i.e.*, ~63) and both the potential for inbreeding and the rate of loss of genetic diversity would be high. However, paternity analysis indicates that the mating strategy of North Atlantic right whales is not based on a dominance heirarchy but rather, a more egalitarian promiscuous system.[16]

Behavioural and reproductive differences among members of the population, and the identification of new adults, suggest that this population is larger than the minimum estimate of 283 in 1997. However, until the location of all mating and nursery grounds are identified, a more precise estimate of the population size will be difficult. Once the existence and location of the mating ground(s) and the alternate summer nursery habitat have been identified, differences in survivorship and reproductive success between the two groups may be considered with regard to habitat quality. The predicted extinction of the North Atlantic right whale within the next 200 years[2] makes intensive monitoring and

the documentation of any change in the size of the population critically important. An accurate estimate of the census population size will require that newly sighted whales be properly identified as calves or novel adults and that Non-BoF calves be genetically identified prior to the migration to the unknown nursery ground (*i.e.*, that they be biopsy-sampled while in the calving ground with their mothers). By combining DNA profiles and demographic data, newly sighted whales and calves may be individually assigned membership to one of the two subpopulations of North Atlantic right whales.

Acknowledgements

This research was supported by an N.S.E.R.C. Scholarship to RCW, N.S.E.R.C. Research funding awarded to BNW, and funding to SK and MB from the National Marine Fisheries Service (USA). Special thanks to Brian Golding and Peter Chu for their assistance with the modelling.

Literature cited

1. New England Aquarium Photoidentification an sighting-resighting database, unpublished. Boston, Massachusetts.

2. Caswell H, Fujiwara M, Brault S (1999) Declining survival probability threatens the North Atlantic right whale. Proceedings of the National Academy Science, USA, 96, 3308-3313.

3. Waldick RC, Brown MW, Kraus SD, Wilson P, White BN (In Prep.) Assessment of the effects of historic bottlenecks on the microsatellite DNA variation in the western North Atlantic right whale (*Eubalaena glacialis*) relative to that in a related species (*Eubalaena australis*).

4. Malik S, Brown MW, Kraus SD, White BN (1999) Assessment of mitochondrial DNA structuring and nursery use in the North Atlantic right whale (*Eubalaena glacialis*). Canadian Journal of Zoology.

5. Kraus SD (1991) Mating strategies in the North Atlantic right whale (*Eubalaena glacialis*). MSc. thesis, University of Massachusetts, Boston.

6. International Union for the Conservation of Nature Redbook. 1993.

7. Chris Slay, Right Whale Consortium Group, Boston, Massachusetts. Personal Communication.

8. Kraus SD, Moore KE, Price CA, Crone MJ, Watkins WA, Winn HE, Prescott JH (1986) The use of photographs to identify individual North Atlantic right whale (*Eubalaena glacialis*). Report of the International Whaling Commission, Special Issue 10, 145-152.

9. Schaeff CM, Kraus SD, Brown MW, White BN (1993) Assessment of the population structure of western North Atlantic right whales (*Eubalaena glacialis*) based on sighting and mitochondrial DNA RFLP data. Canadian Journal of Zoology, 71, 339-345.

10. Kraus SD, Prescott JH, Knowlton AR, Stone GS (1986) Migration and calving of right whales (Eubalaena glacialis) in the western North Atlantic. Report of the International Whaling Commission, Special Issue 10, 139-144.

11. Knowlton AR, Kraus SD, Kenney RD (1994) Reproduction in North Atlantic right whales (*Eubalaena glacialis*). Canadian Journal of Zoology, 72, 1297-1305.

12. Nei M (1987) Molecular Evolutionary Genetics Columbia University Press, New York.

13. Sokal RR, Rohlf FJ (1981) Biometry. 2nd ed. Freeman and Company, New York.

14. Goodman SJ (1997) RST CALC: A collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. Molecular Ecology, 6, 881-885.

15. Waser PM, Strobek C (1998) Genetic signitures of interpopulation dispersal. Trends in Ecology and Evolution, 13, 43-44.

16. Waldick RC, Kraus SD, Brown MW, White BN (In Prep.) Estimating the effective population size and mating system of the endangered North Atlantic right whale using genetic and demographic data.

17. Kraus SD (1990) Rates and potential causes of mortality in North Atlantic right whales (*Eubalaena glacialis*). Marine Mammal Science, 6, 278-291.

18. Kenney RD, Hyman MAM, Owen RE, Scott GP, Winn HE (1986) Estimation of prey densities required by western North Atlantic right whales. Marine Mammal Science, 2, 1-13.

19. Schaeff CM, Kraus SD, Brown MW, Perkins JS, Payne R, White BN (1997) Comparison of North and South Atlantic right whale (*Eubalaena*) genetic variability

using DNA fingerprinting. Canadian Journal of Zoology, 75, 1073-1080.

20. Waples RS (1991) Genetic methods for estimating the effective size of cetacean populations. Report of the International Whaling Commission, Special Issue 13, 279-301.

21. Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology, 7, 639-655.

Chapter four

The use of microsatellite DNA variation for estimating the timing of the loss of genetic variability from the endangered western North Atlantic right whale (*Eubalaena glacialis*).

Abstract

The right whales in the North Atlantic ocean are represented by a small population of 283 identifiable individuals (*Eubalaena glacialis*). The *E. glacialis* population is estimated to have been on the order of 12,000-15,000 individuals prior to the onset of whaling in the 11th century. In contrast, the South Atlantic right whale population (*Eubalaena australis*) is not known to have dropped below several thousand individuals. We tested the hypothesis that the low genetic variation in *E. glacialis* occurred as a result of a recent population decline (19th century) by comparing the levels of variability between *E. glacialis* (N = 209) and *E. australis* (N = 29). A total of 22 microsatellite primer pairs were selected, including 13 developed for other cetacean species, eight developed for *Eubalaena glacialis,* and one set developed for bovids. Microsatellites were not identified in either species for 11 of the primer pairs, however, three heterologous and eight *Eubalaena*-specific loci were polymorphic in *E. australis*. The average number of alleles (A = 7.4 $\pm$ 4.0) and heterozygosity (H = 0.71 $\pm$ 0.2) observed in *E. australis* was similar to that reported for other large mammals, including the North Atlantic fin whale (*Balaenoptera physalus*). In contrast, genetic variability was significantly lower in *E. glacialis* (A = 4.5 $\pm$ 2.5, H = 0.50 $\pm$ 0.1, p = 0.0007 and 0.005, respectively) and only 54% of the 11 loci were polymorphic. Half of these loci had two or three alleles, which differed incrementally by single repeat units. Bottleneck analyses indicate that most of the loss of genetic variation from the *E. glacialis* population occurred over a protracted period (*ca.* 500 years) and not during the most recent population bottleneck (19th century).

Introduction

A widely accepted view among biologists is that the capacity of a population to persist over evolutionary time is related to the level of genetic variation present in that population (Soule and Wilcox 1980; Frankel and Soule 1981; Soule 1986). Over the shorter term, a small population will lose diversity through genetic drift at a rate proportional to its effective population size (*i.e.*, $1/2N_e$ per generation; Nei *et al.* 1975). A population that undergoes a marked reduction in size (population bottleneck) and this, in turn, will be manifested as a reduction in overall levels of heterozygosity within the population. Such a reduction in overall variability may then affect the fitness of a population through the loss of heterosis (see Coulson *et al.* 1998). In humans, for example, the incidence of spontaneous abortion was found to be higher when both fetal alleles matched those of the mother at loci within the Human Lymphocyte Antigen complex (Ober *et al.* 1998).

Members of a small population are more likely to be related to each other than are those within a large population. Consequently, matings among closely-related individuals will be more common, and deleterious recessive alleles are more likely to be expressed in small populations (Frankel and Soule 1981; Lande 1988; Mills and Smouse 1994; Frankham 1995). Inbreeding depression has been documented in several small captive populations (Ralls *et al.* 1979; Lacy *et al.* 1996; Laikre *et al.* 1996), however, the influence of inbreeding depression on small natural populations has not been well established (but see Packer *et al.* 1991; Keller *et al.* 1994; Saccheri *et al.* 1998). Within small populations, the combined effects of limited genetic variability and a small

effective population size (N_e) may exacerbate inbreeding depression, leading to reduced survivorship/reproduction and a further loss of genetic variability (Lande 1991; Lynch 1994).

The highly endangered North Atlantic right whale (*E. glacialis*) has numbered around 300 individuals throughout the past century (Knowlton *et al.* 1994), 283 of which were known to be alive in 1997. Although the population appears to have remained stable throughout much of this century, recent modelling efforts suggest that the growth rate declined from a slightly positive to a negative rate between 1980 and 1994 (Caswell *et al.* 1999). The reasons for the decline remain speculative, however, low genetic variation and inbreeding depression have been suggested as possible influences (Kraus 1990, Knowlton *et al.* 1994; Kenney *et al.* 1995).

Historically, northern right whales were distributed throughout the North Pacific and North Atlantic oceans. Several centuries of commercial whaling severely reduced both the size of these populations and the extent of their ranges. The Eubalaenid whales in the Atlantic, include *E. australis* which is currently distributed throughout much of the South Atlantic ocean and *E. glacialis* which occurs north of the equator where it is largely restricted to the coastal waters off eastern North America. Many similarities exist between the reproductive potential, behaviour, and general natural history of *E. australis* and *E. glacialis* (Payne 1986; Best 1988). The potential for growth by the two populations should be similar, as females have their first calves at *ca.* eight or nine years of age and are capable of producing calves at intervals of four years (Knowlton *et al.* 1994), however, the growth rate reported for *E. australis* is over three-fold greater than

that of *E. glacialis* (1.075 versus 0.976-to-1.025 per year, respectively; Payne 1986; Best 1988; Knowlton *et al.* 1994; Caswell *et al.* 1998).

The key difference in the whaling histories of *E. glacialis* and *E. australis* is that the period of exploitation was much shorter for the latter and the population has remained in the thousands of individuals within several subdivided populations throughout the southern hemisphere (Braham and Rice 1984). In contrast, commercial whaling starting in the 11th century reduced the size of the *E. glacialis* population from an estimated pre-exploitation size of 12,000-15,000 to commercial extinction by the 16th century (Aguilar 1986; Reeves and Mitchell 1986a,b; Gaskin 1987). Around this time, whaling activities by Basque, British, Dutch, and Danish fishermen had spread westwards and were being increasingly directed at the western population. The number of *E. glacialis* harvested increased up until the 18th century, when the population showed signs of depletion throughout most of its range (Reeves and Mitchell 1986a,b). By the end of the 19th century, the entire North Atlantic right whale population had reached commercial extinction and may have been reduced to fewer than 50 animals (Kenney *et al.* 1995; Knowlton *et al.* 1994).

The low levels of variation at minisatellite loci (Schaeff *et al.* 1997) and low mitochondrial haplotypic diversity (Schaeff *et al.* 1993; Schaeff *et al.* 1997; Malik *et al.* In Press) observed in *E. glacialis* are consistent with expectations for a small population that has undergone a bottleneck event. The whaling records suggest two possible scenarios for the historic population decline in *E. glacialis* (Figure 4-1). The first scenario suggests that the populations in the eastern and western parts of the North Atlantic ocean

were distinct and that each experienced a separate size reduction (see Reeves and Mitchell 1986a). Under this scenario, the population in the western North Atlantic would now be represented by the present-day population. The loss of genetic variability would, therefore, be expected to have occurred more recently (*i.e.*, 19th century). The second scenario for the population decline is one in which right whales had been affected throughout the North Atlantic ocean by the 16th century. In this scenario, the loss of variability occurred during a protracted bottleneck event.

To assess the levels of heterozygosity in the two species of right whale and examine possible scenarios for the timing of the loss of variability in *E. glacialis*, we examined genetic variability at microsatellite loci. Microsatellites are short nucleotide repeat units of 100-300 base pairs in length that can undergo high mutation rates (10^{-3} gametes/ generation; Tautz 1989; Weber and May 1989; Bruford and Wayne 1993). New alleles are thought to be acquired through stepwise mutation events where repeat units are incrementally gained or lost (Shriver *et al.* 1993; Goldstein *et al.* 1995; Nauta and Weissing 1996). Because these regions are non-coding, the loss of alleles is heavily influenced by the number of breeders in a population ($1/2N_e$ per generation; Nei *et al.* 1975).

Materials and Methods

Sample collection and DNA extraction

Tissue samples used in this study were collected using biopsy-darting techniques as described in Brown *et al.* (1991). Skin samples for the 209 individual *E. glacialis* used in this study were collected from the Bay of Fundy and the Great South Channel between 1988 and 1998 and from Baccaro Banks off the Southern Scotian Shelf between 1988 and 1992. Twenty-nine skin samples from *E. australis,* collected offshore from the Peninsula Valdes in Argentina were also used in this study (Schaeff *et al.* 1993). DNA was extracted from these samples using phenol-chloroform extraction protocols outlined in Brown *et al.* (1991) and Schaeff *et al.* (1993).

Development and selection of microsatellites

Microsatellite loci containing di-nucleotide repeats (TG_n and TC_n) were isolated from the genome of *E. glacialis* following the protocol outlined in Rassman *et al.* (1991). Forward and reverse primers for the amplification of the microsatellite loci were developed using the program Primer (Lincoln *et al.* 1991) for sequences containing microsatellite regions containing 10 or more repeat units (Waldick *et al.* a, In Press). To compare the levels of genetic diversity between *E. glacialis* and *E. australis,* eight primer pairs were randomly selected from the 18 developed for *Eubalaena* (Waldick *et al.* a, In Press). An additional 14 heterologous primer pairs developed for other taxa were also

screened on *E. glacialis* (Schlotterer *et al.* 1991; Buchanan *et al.* 1996; Valsecchi and Amos 1996).

Polymerase chain reactions were carried out in 10 uL reactions using 20-50 ng of template DNA (0.2 uM of each primer, 20 nM of γ-^{33}P end-labeled primer, 2.0-2.5 mM $MgCl_2$, and the 1x PCR buffer, 0.7 Units *Taq*, and 0.20 mM dNTP; Gibco-BrL). PCR cycles consisted of a 5 mn step at 94° C followed by 25-30 cycles of 30 s at 94° C, 30 s at 50-56° C, and 45 s at 72° C. One final 10 min. extension step at 72° C was added to the end of the reaction. PCR products were separated by size on a 6% denaturing, polyacrylamide sequencing gel and visualized by autoradiography and exposure to phosphoImager screens (Molecular Dynamics, Sunnyvale California). Alleles were defined by their phenotype of a single strong band with two weaker intensity bands that were 2 and 4 bp smaller, respectively.

Statistical analysis

Allele frequency data were compared between taxa and each locus was tested for deviations in genotype frequencies from Hardy-Weinberg equilibrium using a 95% confidence interval established by performing 10,000 bootstraps and permutations on the allele frequency data (GenePop v.3; Raymond and Rousset 1995). Populations that have recently experienced a bottleneck event, may also show a residual "heterozygote excess" for several generations due to the loss of rare alleles, which have little influence on the overall heterozygosity (Cornuet and Luikart 1996; Luikart and Cornuet 1998; Luikart *et al.* 1998). This heterozygote excess occurs as a result of the more rapid rate at which

alleles are lost from a small population than is heterozygosity. This condition persists until a new equilibrium is established between the rate of loss through random genetic drift and the accumulation of new alleles through mutation. Populations that have undergone a recent bottleneck event will not have re-established equilibrium between genetic drift and mutation. As a result, such populations will show an excess of heterozygosity. To determine if significantly more loci show an excess of heterozygosity than would be expected by chance at mutation-drift equilibrium, expected heterozygosities are generated for the population data set and tested against the observed heterozygosity for each locus. For microsatellite loci, mutation is best modelled according to a 'two phase model' in which most mutations occur as single-step events, and the remainder (5-10%) occurring as multiple-step events (Luikart and Cornuet 1998). At mutation-drift equilibrium, *ca.* half of the microsatellite loci evaluated will be expected to show a slight excess of heterozygosity. The bottleneck test then determines if the ratio of the number of loci with heterozygote excess to those with deficiencies differs significantly from that expected by chance under mutation-drift equilibrium (Sign test; Bottleneck program, Luikart and Cornuet 1998). Differences in the number of polymorphic loci, average heterozygosity, and allelic diversity were directly compared between the two species of Eubalaenid whale (t-test; Sokal and Rohlf 1981).

Results

Characterization of microsatellite loci

In total, eight *Eubalaena*-specific primer sets (Waldick *et al.* In press) and 13 microsatellite primer sets developed for other cetacean species (Schlotterer *et al.* 1991; Buchanan *et al.* 1996; Valsecchi and Amos 1996) were screened for polymorphisms in *E. glacialis* and *E. australis.* Eleven of the cross-species microsatellite primer sets did not amplify identifiable products in *E. australis* or *E. glacialis.* All were excluded from the study. These included primer pairs developed for the beluga whale (DlrFCB 1,4,5,6,13,16,17; Buchanan *et al.* 1996) and four pilot whale primers (415/416, 417/418, 21P, and 30P ; Schlotterer *et al.* 1991; Valsecchi and Amos 1996). Of the eight *E. glacialis*-derived microsatellite loci studied, seven were polymorphic in *E. australis* while only three were polymorphic in *E. glacialis* (Table 4-1).

An additional polymorphic locus was identified in both *Eubalaena* species using a heterologous microsatellite locus associated with the insulin-like growth factor 1 gene (IgF-1). This microsatellite locus consists of a series of dinucleotide repeats in the 5' portion of the functional gene, and has been shown to be conserved in *Bos taurus* (cow), *Sus scrofa* (boar; Kirkpatrick 1992), and more recently in harbour porpoise (*Phocoena phocoena*; Andersen *et al.* 1997). This locus was one of the two most heterozygous loci identified in *E. glacialis* (Table 4-2).

Table 4-1. Allelic diversity, locus-specific heterozygosity (H), and an evaluation of the informativeness of microsatellite loci in *E. glacialis* (*Eg*) and *E. australis* (*Ea*).

Locus	Sp.	No. Alleles	Ho[1]	He[2]	PE	Chrms[3] Screened[3]
1P†	*Ea*	94	0.84	0.85	0.50	58418
	Eg		0.43	0.47	0.18	
37M†	*Ea*	113	0.90	0.88	0.73	58418
	Eg		0.51	0.54	0.26	
94M†	*Ea*	31	0.60	0.50	0.20	5858
	Eg		-	-	-	
IgF	*Ea*	1310	0.96	0.91	0.80	42418
	Eg		0.78	0.76	0.57	
Rw217	*Ea*	31	0.48	0.40	0.20	5858
	Eg		-	-	-	
Rw219	*Ea*	41	0.45	0.65	0.37	5860
	Eg		-	-	-	
Rw17	*Ea*	21	0.28	0.29	0.12	5858
	Eg		-	-	-	
Rw18	*Ea*	145	0.69*	0.86	0.71	58418
	Eg		0.51	0.51	0.26	
Rw21	*Ea*	61	0.67	0.80	0.59	5858
	Eg		-	-	-	
Rw26	*Ea*	113	1.0	0.89	0.75	58418
	Eg		0.44	0.42	0.16	
Rw31	*Ea*	75	0.89	0.79	0.57	58418
	Eg		0.48	0.50	0.29	
Average	*Ea*	7.4 + 4.0	0.71 $\pm$ 0.2		Range 0.12-0.80	
	Eg	2.9 + 2.5	0.52 $\pm$ 0.1		0-0.57	

Primers indicated as 'RW" are Eubalaena-derived (Waldick et al. In Press). [1]Observed Heterozygosity, [2] Expected Heterozygosity, [3] Number of chromosomes screened. *Heterozygous excess $p < 0.05$. Informativeness is expressed as the probability of exclusion (PE) for each species.†Schlotterer *et al.* 1991.

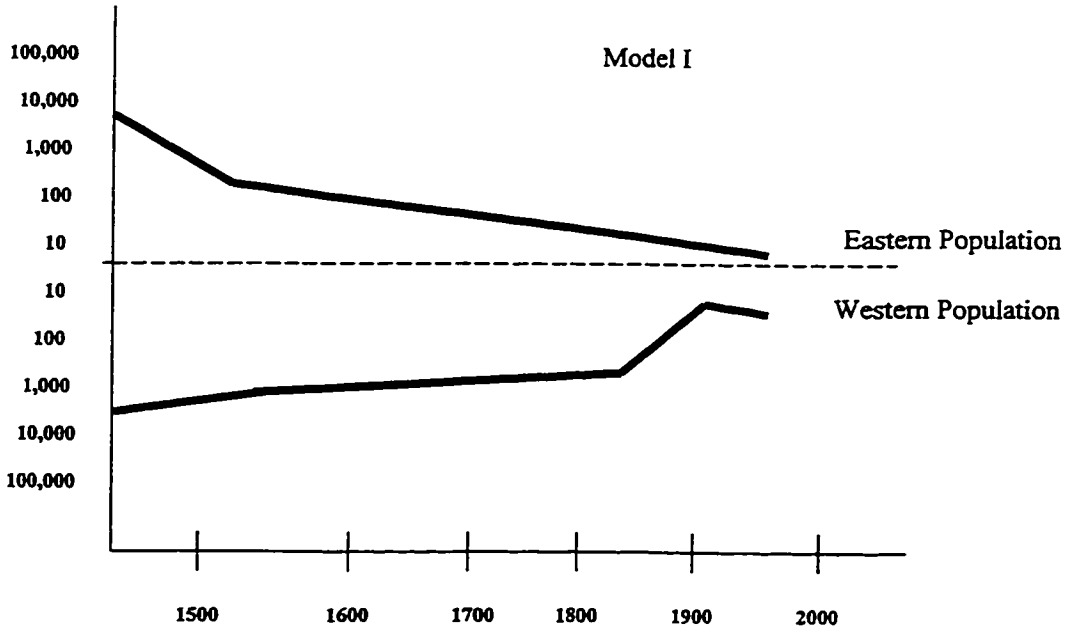
Table 4-2. Sizes (bp) and frequencies of microsatellite alleles at nine polymorphic loci in *E. australis* and *E. glacialis*.

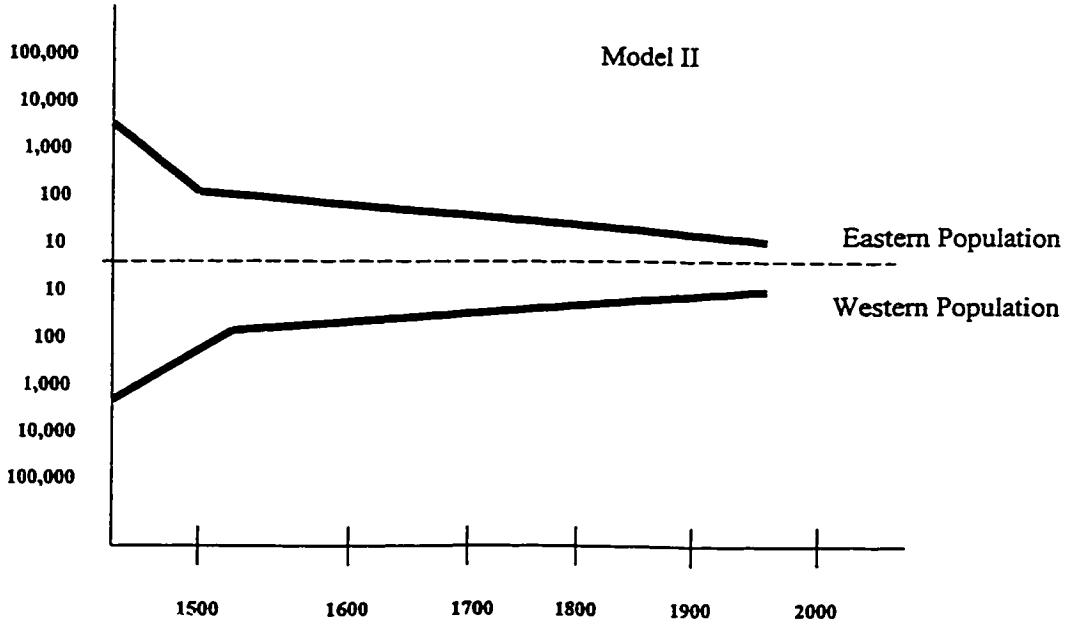
Allele (bp)	*Eg*	*Ea*	Allele (bp)	*EgTabl*	*Ea*	Allele (bp)	*Eg*	*Ea*
1P			**IgF**			**37M**		
125		0.14	**149**		0.04	**189**		0.07
127		0.20	**151**	0.03	0.09	**191**		0.12
129		0.10	**153**	0.02	0.11	**193**		0.09
133	0.002	0.02	**155**	0.07	0.09	**195**		0.07
135	0.618	0.04	**157**	0.05	0.11	**197**		0.05
137	0.38	0.16	**159**	0.41	0.09	**199**	0.39	0.03
139		0.26	**161**	0.15	0.14	**201**	0.55	0.14
143		0.04	**163**	0.03	0.11	**203**	0.06	0.03
147		0.04	**165**	0.21	0.12	**205**		0.26
			167	0.002	0.02	**207**		0.09
94M			**169**	0.03		**209**		0.05
195		0.02	**171**		0.02			
197	1.0	0.59	**183**		0.02	**Rw21**		
201		0.39	**185**		0.04	**313**		0.02
						317	1.0	0.19
Rw26			**Rw217**			**321**		0.14
159		0.03	**167**	1.0	0.78	**323**		0.20
161		0.12	**169**		0.13	**325**		0.37
163	0.28	0.05	**171**		0.08	**327**		0.04
165	0.71	0.05						
167	0.01	0.04	**Rw18**			**Rw31**		
169		0.11	**185**		0.14	**116**		0.22
171		0.13	**187**	0.05		**118**		0.02
173		0.14	**189**		0.05	**120**		0.19
175		0.07	**191**	0.05	0.09	**122**	0.17	0.33
177		0.21	**193**	0.02	0.31	**124**		0.15
179		0.05	**195**	0.66	0.10	**126**		0.07
			197	0.22	0.12	**128**	0.04	
Rw219			**199**		0.03	**130**	0.67	0.02
85		0.48	**201**		0.02	**132**	0.09	
87		0.27	**203**		0.02	**134**	0.03	
89	1.0	0.20	**209**		0.03			
91		0.05	**233**		0.02			

			239	0.02
Rw17			**245**	0.03
249		0.16	**247**	0.02
251	1.0	0.84		

*Loci classified as monomorphic were screened on N = 58 chromosomes. The number of chromosomes screened was N = 58 for *E. australis* (*Ea)* and N = 418 for *E. glacialis**.

Figure 4-1. Two possible models for the historic changes in population size for *E. glacialis* in the North Atlantic ocean. The entire North Atlantic population is represented by the two halves of each figure. The eastern population is depicted in the top half of the figure and the western population is represented by the lower half of the diagram. A) Model I- The depletion of the eastern population had occurred by the 16th century, coincident with increasing pressure on the western population. By the 19th century, an extreme population bottleneck occurred as the result of the loss of most of the western population. A 29% loss of heterozygosity due to this bottleneck event would have resulted in an $N_e \approx 20$ during the 19th century. B) Model II- Whaling pressures affected the western and eastern populations concurrently. By the 16th century, whaling pressures had affected the population throughout the North Atlantic ocean, reducing the overall population size by an order of magnitude. The population persisted at a reduced size in the western part of the distribution into this century. The minimum N_e necessary to account for the loss of 29% of historic heterozygosity levels over a period of 400 years is *ca.* 80.


Model I
100,000
10,000
1,000
100
10
10
100
1,000
10,000
100,000
Eastern Population
Western Population
1500
1600
1700
1800
1900
2000


Model II
100,000
10,000
1,000
100
10
10
100
1,000
10,000
100,000
Eastern Population
Western Population
1500
1600
1700
1800
1900
2000

Interspecific comparison of polymorphism and genetic diversity

Although none of the microsatellite primers used were derived from *E. australis*, and the number of these samples was smaller (N = 29) than that of *E. glacialis* (N = 209), a larger number of microsatellite loci were polymorphic in *E. australis*. Heterozygosity at the 11 microsatellite loci studied was also significantly greater in *E. australis* than in *E. glacialis* ($p < 0.005$) and both the average number of alleles and the range in allele size was larger in small populations (Table 4-2). Although some overlap in allele sizes existed between the two species, allele frequencies differed significantly between the two species (Fisher exact test, $p < 0.001$).

Overall, *E. australis* was found to have many low frequency alleles at most microsatellite loci while *E. glacialis* generally had 1-2 common alleles and a few rare alleles. The standard deviation in allele size and average allelic diversity (A) at these loci was also greater in *E. australis* ($A = 7.4 \pm 4.0$) than *E. glacialis* (2.9 ± 2.5, $p < 0.0001$; Table 4-1). While *E. australis* had an average of seven alleles/locus, only two loci had five or more alleles in *E. glacialis* (rw31 and IgF-1). The more equitable distribution of allele frequencies, and the higher number of alleles in *E. australis*, is reflected in the greater overall Probability of Exclusion of non-parental adults (Table 4-1) and the higher heterozygosity (H) in *E.australis* relative to *E. glacialis* ($H = 0.71 \pm 0.22$ and 0.52 ± 0.12; $p = 0.03$)

Bottleneck analysis

The number of loci with heterozygosity excess/deficiency was assessed for the

microsatellite loci using a combined model that incorporates both the stepwise and infinite alleles models of mutation (*i.e.*, Two-phase mutation model in the Bottleneck test; Luikart and Cornuet 1997). The ratio of heterozygote excess to deficiency (5:4) in *E. glacialis* was the same as that expected for a population in mutation-drift equilibrium under the Two-phase mutation model ($p = 0.57$).

Discussion

The endangered North Atlantic right whale (*E. glacialis*) has levels of genetic variation at microsatellite loci that are much lower than those of its close relative (*E. australis*), other cetacean species, and a variety of other large, long-lived mammals (*e.g.*, Paetkau and Strobeck 1994; Roy *et al.* 1994; Allen *et al.* 1995; Buchanan *et al.* 1996; Richard *et al.* 1996; Palsbøll *et al.* 1997; Berubé *et al.* 1998). A new explanation has been proposed to account for low genetic diversity in species/populations with no history of population bottleneck events ('Genome-wide selective sweeps'; see Amos and Harwood 1998). Under this hypothesis, strong positive selection acts on particular parts of the genome and results in the rapid loss of variability throughout the genome. Although such a mechanism could account for low diversity at some loci in the nuclear genome, it is unlikely to affect most loci and would not affect mtDNA diversity. The low mtDNA diversity in *E. glacialis* is consistent with a species that has experienced a population bottleneck event, suggesting that the low variability within the nuclear genome is also associated with a bottleneck event. *Eubalaena glacialis* is also known to have gone through two reductions in number during the past 700 years, as such, much of the low allelic diversity is expected to be related to historically low (or fluctuating) population sizes.

Although the allelic diversity and heterozygosity in *E. australis* was substantially higher than that in *E. glacialis*, these levels were not unusually high among cetaceans and were similar to those reported for other large-bodied cetacean populations of a similar population size, including, for example, the North Atlantic fin whale, *Balaenoptera*

physalus (A = 7.4 versus 7.0 and H = 0.71 versus 0.67, respectively; Berubé *et al.* 1998). In contrast, the low genetic variability observed in *E. glacialis* was consistent with those reported for other natural populations that have undergone population bottlenecks (Taylor *et al.* 1994; Houlden *et al.* 1996; Luikart *et al.* 1998). For example, a 41% loss of heterozygosity was reported in a wombat population (*Lasiorhinus krefftii*) that was reduced in number from 1000 individuals to 25 individuals within a period of 120 years (Taylor *et al.* 1994). The 29% difference in heterozygosity between *E. australis* and *E. glacialis* is modest relative to that reported in this wombat population, nevertheless, the allelic diversity and heterozygosity in the post-bottleneck wombat population are consistent with the levels presently observed in *E. glacialis*. The genetic variability observed in *E. australis*, on the other hand, is consistent with that predicted under the Stepwise Mutation Model for a population with an N_e in the thousands, and a rate of mutation of 10^{-4} gametes per generation (Shriver *et al.* 1993).

Given that the current *E. australis* population in the South Atlantic ocean is of a similar size to that of the pre-bottleneck estimates for *E. glacialis* (Braham and Rice 1984; Reeves and Mitchell 1986a,b; Gaskin 1987), the heterozygosity of the former may be used as an estimate of pre-bottleneck heterozygosity levels (H_o) in *E. glacialis*. Using an H_o of 0.71 and the current level of heterozygosity in *E. glacialis* (H_t = 0.50), a 29% loss of heterozygosity has occurred in the population. The relative time of the bottleneck may be estimated using the equation: $H_t = H_o (1- 1/2N_e)^t$, where 't' is time in generations (Crow and Kimura 1970). If N_e had been as low as 50 during the 19th century, only 10% of the original level of heterozygosity would have been lost. If such an extreme bottleneck event

occurred 10 generations ago, bottleneck analysis would have identified an excess of loci with an excess of heterozygosity (*i.e.*, within 10-to-200 generations; Cornuet and Luikart 1996; Luikart and Cornuet 1998; Luikart *et al.* 1998). Power to detect severe population bottlenecks such as that proposed for the North Atlantic right whale is reasonable (~80%). Whaling records from the 18th and 19th centuries also fail to provide support for the hypothesis that the effective number of breeders was as low as 20 individuals. Moreover, small sampling effects associated with such a severe bottleneck event would be expected to result in the random retention of alleles within a population. The random passing of alleles through a bottleneck event would be expected to result in allele frequency distributions composed of a random sub-sampling of the alleles from the pre-bottleneck population. In *E. glacialis,* most microsatellite loci were monomorphic or had two or three alleles differing in size by a single repeat unit; this suggests that some of these loci may have been fixed for a single allele, but that enough time has passed since the decline for alleles to become fixed or new mutations to have accumulated.

Given that genetic variability was low prior to the population bottleneck of the 19th century, the loss may be attributable to a more historic bottleneck event, perhaps one associated with the first reported population decline. A more probable model for the 29% loss of heterozygosity based on the amount of variation lost, is one in which a protracted period of exploitation (including the near extirpation of the eastern population) reduced the N_e. The loss of variability would have been intensified by a more recent population bottleneck (19th century). For example, most of the 29% loss of heterozygosity is accounted for in *E. glacialis* in a scenario in which the N_e for the western North Atlantic

had a harmonic mean of *ca.* 500 for five centuries (13th-to-18th century) but was reduced to 50 throughout the 19th century.

Additional evidence for a more historic date for the loss of genetic variability in *E. glacialis* is provided by a study of mitochondrial haplotype diversity in present-day *E. glacialis,* and samples from whales harvested 90 years ago (Rosenbaum *et al.* In Press; Malik *et al.* 1999). Four of the mitochondrial haplotypes present in the whale samples from one century ago are represented in the current *E. glacialis* population. As these older samples include whales harvested from different parts of the North Atlantic ocean, the absence of some haplotypes suggests that mitochondrial diversity had already been reduced throughout this population prior to the 19th century (*i.e.*, pre-exploitation diversity was low). These findings indicate that a population bottleneck had reduced the genetic variation in *E. glacialis* throughout its distribution prior to the 19th century. Currently, analyses of the levels of mitochondrial and microsatellite diversity are being assessed in ancient baleen samples dating to the 16th century. These samples will identify heterozygosity levels prior to the most recent bottleneck event (19th century) and confrim the extent to which variability had been lost by the 16th century.

The failure of the *E. glacialis* population to show any signs of population growth during the past century has been hypothesized as being the result of inbreeding depression (Schaeff *et al.* 1997). The effects of inbreeding on a population are acute, however, and would be expected to be expressed as reduced survivorship and reproductive fitness shortly after a population bottleneck event (Lynch *et al.* 1996). Given that genetic variability was lost over several centuries, and that the most recent bottleneck event occurred over a

century ago, it is unlikely that inbreeding depression would still have a strong effect on this population. Given the relatively recent onset of the negative growth rate, and the antiquity of the bottleneck event, it is unlikely that inbreeding depression is the cause of the recent population decline. However, the historic loss of allelic diversity may have resulted in the loss of adaptive potential or heterotic effects.

Recent modelling based on 17 years of sighting-resighting data indicates that the growth rate for this population was positive until very recently (Knowlton *et al.* 1994; Caswell *et al.* 1999). The recent decline appears to be a consequence of a lowering in individual survivorship that is not compensated for by calf production. The low calf production may be associated with the low genetic variability in this population. If, for example, genetic variability at loci within the Major Histocompatibility Complex is low, the likelihood of both the fetus and the mother carrying complimentary alleles may be high. In humans, fetuses that inherit a paternal allele that does not differ from either of those present in the mother are more likely to be aborted than those inheriting a unique allele (Ober *et al.* 1998). Any such mechanism that increases the incidence of spontaneous abortion in *E. glacialis* may explain the low incidence of multiple calvings by females in this species.

Given that genetic variability may have been low for several centuries, the low calf production in *E. glacialis* has probably been a characteristic of this population for several centuries. The ability of this species to persist for several centuries despite its low rate of calf production is remarkable, particularly given that whaling continued into the 19th century and that the population had already lost much of its genetic variability by that time.

Any effects of an increased incidence of inbreeding or the loss of heterosis associated with the historic bottleneck have affected this population for over 100 years and are unlikely to be the cause of the recent population decline. The main limitations for growth by this population probably include those intrinsic factors associated with low calf production, and those extrinsic factors that may be increasing mortality, including habitat disturbances and human-related mortalities (Kraus 1990).

Acknowledgments

This research was supported by an N.S.E.R.C. Scholarship to RCW, N.S.E.R.C. Research funding awarded to BNW, and funding to SK and MB from the National Marine Fisheries Service (USA). Additional support was received from World Wildlife Fund Canada. Thanks to Cindy Thomlinson, Lisle Gibbs, and Jim Quinn for helpful suggestions on an early draft of this manuscript.

Literature Cited

Aguilar A (1986) A review of old Basque whaling and its effect on right whales (*Eubalaena glacialis*) of the North Atlantic. Reports of the International Whaling Commission (Special Issue 10), 191-199.

Allen PJ, Amos W, Pomeroy PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. Molecular Ecology, 4, 653-662.

Amos W (1996) Levels of genetic variability in cetacean populations have probably changed little as a result of human activities. Reports of the International Whaling Commission (Special Issue 46), 657-658.

Amos W, Harwood J (1998) Factors affecting levels of genetic diversity in natural populations. Philosophical Transactions of the Royal Society of London, 179-186.

Andersen LW, Holm L, Siegismund HR, Clausen B, Kinze CC, Loeschcke V (1997) A combined DNA-microsatellite and isozyme analysis of the population structure of the harbour porpoise in Danish waters and west Greenland. Heredity, 78, 270-276.

Berubé M, Aguilar A, Dendanto D, Larsen SF, Notarbartolo di Sciara G, Sears R, Sigurjønsson J, Urban-R J, Palsbøll PJ (1998) Population genetic structure of North Atlantic, Mediterranean sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. Molecular Ecology, 7, 585-599.

Best PB (1988) Right whales *Eubalaena australis* at Tristan da Cunha. A clue to the non-recovery of depleted stock? Biological Conservation, 46, 23-51.

Braham HW, Rice DW (1984) The right whale, *Balaena glacialis*. Marine Fisheries Review, 46, 38-53.

Brown MW, Kraus SD, Gaskin DE (1991) Reaction of North Atlantic right whales (*Eubalaena glacialis*) to skin biopsy sampling for genetic and pollutant analysis. Report International Whaling Commission (Special Issue 13), 81-90.

Bruford MW, Wayne RK (1993) Microsatellites and their application to population genetics. Current Opinion in Genetics and Development, 3, 939-943.

Buchanan FC, Friesen MK, Littlejohn RP, Clayton JW (1996) Microsatellites for the beluga whale *Delphinapterus leucas*. Molecular Ecology, 5, 571-575.

Caswell H, Fujiwara M, Brault S (1999) Declining survival probability threatens the North Atlantic right whale. Proceedings of the National Academy Science, USA, 96, 3308-3313.

Cornuet JM, Luikart G (1996) Description and evaluation of two tests for detecting recent bottlenecks. Genetics, 144, 2001-2014.

Coulson TN, Pemberton JM, Albon SD, Beaumont M, Marshall TC, Slate J, Guinness FE, Clutton-Brock TH (1998) Microsatellites reveal heterosis in red deer. Proceedings of the Royal Society, London, 265, 489-495.

Crow JF, Kimura M (1970) An introduction to population genetics theory. Harper and

Row, New York.

Frankel OH, Soule ME (1981) Conservation and evolution. Cambridge University Press, Cambridge, United Kingdom.

Frankham R (1995) Inbreeding depression: a threshold effect. Conservation Biology, 9, 792-799.

Gaskin DE (1987) Updated status of the right whale, *Eubalaena glacialis*, in Canada. Canadian. Field Naturalist, 101, 295-309.

Goldstein DB, Ruiz Linares A, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics, 139, 463-471.

Houlden BA, England PR, Taylor AC, Greville WD, Sherwin WB (1996) Low genetic variability of the koala *Phascolarctos cinereus* in south-eastern Australia following a severe population bottleneck. Molecular Ecology, 5, 269-281.

Keller LF, Arcese P, Smith JNM, Hochachka WM, Stearns SC (1994) Selection against inbred song sparrows during a natural population bottleneck. Nature, 372, 356-357.

Kenney RD, Winn HE Macaulay MC (1995). Cetaceans in the Great South Channel, 1979-1989: right whale (*Eubalaean glacialis*). Continental Shelf Research ,15, 385-414.

Kirkpatrick BW (1992) Identification of a conserved microsatellite site in the porcine and bovine insulin-like growth factor-I gene 5' flank. Animal Genetics, 23, 543-548.

Knowlton AR, Kraus SD, Kenney RD (1994) Reproduction in North Atlantic right whales (*Eubalaena glacialis*). Canadian Journal of Zoology, 72, 1297-1305.

Kraus SD (1990) Rates and potential causes of mortality in North Atlantic right whales (*Eubalaena glacialis*). Marine Mammal Science, 6, 278-291.

Lacy RC, Alaks G, Walsh A (1996) Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. Evolution, 50, 2187-2200.

Laikre L, Andren R, Larsson H, Ryman N (1996) Inbreeding depression in brown bear. Biological Conservation, 76, 69-72.

Lande R (1988) Genetics and demography in biological conservation. Science, 241, 1455-1459.

Lande R (1991) Applications of genetics to management and conservation of cetaceans. Report of the International Whaling Commission (Special Issue 13), 301-311.

Lincoln SE, Daly JJ, Lander ES (1991) Primer: a computer program for automatically selecting PCR primers. Version 0.5.

Luikart G, Cornuet J (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology, 12, 228-237.

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology, 7, 963-974.

Lynch M, Conery J, Burger R (1996) Mutation accumulation and the extinction of small populations. American Naturalist, 146, 489-518.

Malik S, Brown MW, Kraus SD, White BN (In Press) Assessment of mitochondrial DNA structuring and nursery use in the North Atlantic right whale (*Eubalaena glacialis*). Canadian Journal of Zoology.

Malik S, Brown MW, Kraus SD, White BN (In Press) Analysis of mitochondrial DNA diversity within and between North and South Atlantic right whales. Marine Mammal Science.

Mills SL, Smouse PE (1994) Demographic consequences of inbreeding in remnant populations. American Naturalist, 144, 412-431.

Nauta MJ, Weissing FJ (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics, 143, 1021-1032.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution, 29, 1-10.

Ober C, Hyslop T, Elias S, Weitkamp LR, Hauck WW (1998) Human leukocyte antigen matching and fetal loss: results of a 10 year prospective study. Human Reproduction, 13, 33-38.

Packer C, Pusey AE, Rowley H, Gilbert DA, Martenson J, O'Brien SJ (1991) Case study of a population bottleneck: lions of the Ngorongo Crater. Conservation Biology, 5, 219-230.

Paetkau D, Strobek C (1994) Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology, 3, 489-495.

Payne R (1986) Long term behavioral studies of the Southern right whale (*Eubalaena australis*). Reports of the International Whaling Comm. (Special Issue 10), 161-167.

Palsbøll PJ, Berubé M, Larsen AH, Jørgensen H (1997) Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. Molecular Ecology, 6, 893-895.

Ralls K, Brugger K, Ballou JD (1979) Inbreeding and juvenile mortality in small populations of ungulates. Science, 206,1101-1103.

Rassman K, Schlotterer C, Tautz D (1991) Isolation of simple-sequence loci for use in polymerse chain reaction-based DNA fingerprinting. Electrophoresis, 12, 113-118.

Raymond M, Rousset F (1995) Genepop (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86, 248-249.

Reeves RR, Mitchell E (1986a) American pelagic whaling for right whales in the North Atlantic. Report of the International Whaling Commission (Special Issue 10), 201-213.

Reeves RR, Mitchell E (1986b) The Long Island, New York, Right Whale Fishery:1650-1924. Report of the International Whaling Commission (Special Issue 10), 201-213.

Richard KR, Whitehead H, Wright JM (1996) Polymorphic microsatellites from sperm whales and their use in the genetic identification of individuals from naturally sloughed pieces of skin. Molecular Ecology, 5, 313-315.

Rosenbaum HC, Egan MG, Clapham PJ, Brownell Jr. RL, Malik S, Brown M, White BN, Walsh P, DeSalle R (In Press) Assessing a century of genetic change in North Atlantic right whales (*Eubalaena glacialis*). Proceedings of the National Academy Science.

Roy MS, Geffen E, Smith D, Ostrander EA, Wayne RK (1994) Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Molecular Biology and Evolution, 11, 553-570.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. Nature, 392, 491-494.

Schaeff CM, Kraus SD, Brown MW, White BN (1993) Assessment of the population structure of western North Atlantic right whales (*Eubalaena glacialis*) based on sighting and mitochondrial DNA RFLP data. Canadian Journal of Zoology, 71, 339-345.

Schaeff, CM, Kraus SD, Brown MW, Perkins JS, Payne R, White BN (1997) Comparison of genetic variability of North and South Atlantic right whales (*Eubalaena*), using DNA fingerprinting. Canadian Journal of Zoology, 75, 1073-1080.

Schlotterer C, Amos B, Tautz D (1991) Conservation of polymorphic simple sequence loci in cetacean species. Nature, 354, 63-65.

Shriver MD, Jin L, Charkraborty R, Boerwinkle E (1993) VNTR allele frequency distributions under the stepwise mutation model: a computer simulation approach. Genetics, 134, 983-993.

Sokal RR, Rohlf FJ (1981) Biometry. 2nd ed. Freeman and Company, New York.

Soule ME (1986) Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, Massachusetts.

Soule ME, Wilcox BA (1980) Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research, 17, 6463-6471.

Taylor AC, Sherwin WB, Wayne RK (1994) Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorhinus krefftii*. Molecular Ecology, 3, 277-290.

Valsecchi E, Amos W (1996) Microsatellite markers for the study of cetacean populations. Molecular Ecology, 5, 151-156.

Waldick RC, Brown MW, Wilson PJ, Kraus SD, White BN (In Press) Characterisation and Isolation of microsatellite loci from the endangered North Atlantic right whale.

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American Journal of Human Genetics, 44, 388-396.

Chapter five

Analysis of the effective population size and mating system of the endangered North Atlantic right whale using genetic and demographic data.

Abstract

Inbreeding associated with small population size has been hypothesized as a potential reason for low calf production in the North Atlantic right whale, however, very little is known about the mating system of this species. To document the occurrence of inbreeding and provide estimates of the effective number of breeders in the population, the mating system was evaluated through paternity and kinship analyses. Microsatellite-based genetic profiles for 209 whales and field observations for 388 whales were used to assess the reproductive success of adult males and females during a 17-year period. Within the 1997 population, 74% of the sexually mature females had produced at least one calf from 1980 to 1997 but only 55% of these females produced multiple calves. Paternity analysis using exclusionary and likelihood techniques identified putative fathers for 39 of 62 calves tested, 24 of which were assigned with a match probability > 80%. The relatedness coefficients observed between mating pairs was similar to the distribution expected among unrelated whales (R_{xy} = -0.035 $\pm$ 0.28 versus 0.0007 $\pm$ 0.24) and less than that among first-order relatives (0.24 $\pm$ 0.23). The distribution of males assigned 1, 2, 3, or 4 paternities was tested relative to that expected under three dominance hierarchies and random mating. The male reproductive skew during the 17-year study period was less extreme than that expected if only 50% or fewer of the males were siring most of the calves (*i.e.*, dominance polygyny; $p < 0.05$). The high proportion of males that were assigned a single calving event in the paternity analysis is consistent with random mating wherein ~60% of males sire offspring. The estimates of the effective population size ranged from 103 to 154 and the ratio of N_e:N was similar to that reported

in other mammal populations. The low realized reproductive success among adult females is considered with regard to low levels of genetic variability and genetic structuring within the population.

Introduction

Small, isolated populations undergo higher rates of inbreeding than larger populations (Frankham 1995, 1997). Consequently, estimates of the effective number of breeders (*i.e.*, effective population size, N_e; Nei 1987) are relevent for assessing the status of a population. The retention of genetic variability within a population is dependent on N_e, a value that is often 50% or less of the census population size (Frankham 1995). N_e is influenced by the mating system of a species, fluctuations in population size, and skewed sex ratios, however, it is variance in lifetime reproductive success that tends to have the greatest effect on N_e (Waples 1991; Nunney and Elam 1994; Rockwell and Barrowclough 1995). As a mating system deviates from random mixing and N_e becomes smaller, a population may be subject to inbreeding depression which may be manifest as a reduced number or lower quality of offspring (Ralls *et al.* 1979; Charlesworth and Charlesworth 1987; Lacy *et al.* 1996).

Multiple generations of monitoring are necessary to collect the information on the lifetime reproductive success of individuals are required to estimate N_e (Barrowclough and Rockwell 1993). Such long-term studies are expensive, particularly for long-lived species, therefore, molecular-genetic analyses are being used increasingly to establish and confirm parentage in natural populations (Chakraborty *et al.* 1988; Queller *et al.* 1993; Keane *et al.* 1997; Marshall *et al.* 1998). One class of highly polymorphic markers that are frequently used in parentage and kinship studies are microsatellite loci (Bruford and Wayne 1993; Queller *et al.* 1993; Double *et al.* 1997; Parker *et al.* 1998). These loci are particularly well-suited for kinship studies because they are distributed throughout the

genome of vertebrates, are non-coding, and follow a Mendelian inheritance pattern (Tautz 1989; Schlotterer *et al.* 1991; Bruford and Wayne 1993). Genetic profiles based on microsatellite loci provide an alternate data set for the identification of individuals, mating system studies, and for the estimation of population size (Amos *et al.* 1991; Richard *et al.* 1996; Clapham *et al.* 1997; Palsbøll *et al.* 1997). Molecular-genetic approaches are especially informative for the study of species like the cetaceans, which are difficult to observe due to their secretive behaviors and large ranges. The status and size of many cetacean populations has not been well established, therefore, molecular-genetic analysis can provide new insights into the size of a population, the rate at which genetic variability is lost, and the degree of inbreeding. When used in combination with long-term monitoring data, this information may be used to refine population size estimates, confirm mating systems (through parentage analysis), and document inbreeding. In contrast to the intensive efforts necessary to monitor and identify whales visually, genetic profiles may be completed relatively quickly using small amounts of DNA amplified by the polymerase chain reaction (see Palsbøll *et al.* 1997).

In this study, we combine 17 years of field observations of the North Atlantic right whale (*Eubalaena glacialis*) with microsatellite-based genetic data to assess adult reproductive success and estimate N_e. Monitoring within the known calving and nursery grounds has identified 92 breeding females and most of the calves they have produced since 1980 (New England Aquarium catalogue).The population is estimated to consist of a minimum of 283 individuals, *ca.* 200 of which are adults ($\geq$ nine years; Knowlton *et al.*

1994), making *E. glacialis* not only one of the best studied of all large-bodied cetaceans but also one of the most endangered. The population growth rate for *E. glacialis* was low but positive during the 1980s (1.025 per year) but has declined in recent years to a negative level (0.975 per year; Caswell *et al.* 1999). During the past 17 years, the *ca.* 100 adult females in the population have produced an average of 11 calves per year (NEAq right whale catalogue). Each female is capable of producing one calf every four years (Knowlton *et al.* 1994), which means that the population could potentially realize an average of 25 births per year. At this rate, the population growth rate would be 1.075 per year, the rate observed in the related South Atlantic right whale (*E. australis*; Payne *et al.* 1990; Burnell 1998).

Low levels of genetic variability in *E. glacialis* (Schaeff *et al.* 1997; Malik *et al.* 1999; Waldick *et al.* In Prep.) and high relatedness among unrelated individuals have led to the suggestion that inbreeding depression may be influencing the low reproductive success of females (Schaeff *et al.* 1997). However, as the population is thought to have lost genetic variability over a century ago (Waldick *et al.* In Prep), the low calf production may be associated with low levels of genetic variability. Given that the identities of 92 reproductive females and their calves are known, molecular-genetic paternity analysis may be used to directly assess the level of inbreeding among mating pairs.

A behavioural study of apparent mating groups consisting of multiple males and a single focal female suggests that males compete for access to females (Kraus 1991). Such a mating strategy may result in a relatively small number of males being able to breed

successfully, consequently, variance in male reproductive success may be high in this population. Individual-specific microsatellite DNA profiles established for 209 animals and the known reproductive histories of all adult females that were alive from 1980 to 1997 were used to assess paternity and kinship among subsets of the population. These data were then used to: (1) identify those females that are not achieving their reproductive potential, (2) establish the existence of a dominance polygyny mating system, (3) examine levels of inbreeding by evaluating the degree of relatedness among breeding pairs, and (4) estimate N_e using both the variance in male and female reproductive success and the observed changes in allele frequencies over time.

Methods

Sighting-resighting records based on individual photo-identifications have been catalogued for 388 whales identified between 1980 and 1997 (NEAq catalogue). The gender of most of these whales (320/388) has been determined through behavioural or genetic gender methods (Knowlton *et al.* 1994; Brown *et al.* 1994; Malik *et al.* 1999). Biopsy-sampling of known whales since the 1980s has resulted in the collection of 484 samples from an estimated 283 living and dead whales. The 209 whales included in the present study, which include 65% of the population known to be alive in 1997, have been genotyped at 9 microsatellite loci (Waldick et al. In Press).

Reproductive Success of Females

Monitoring of females within known calving and nursery grounds throughout the 17 year period has identified 208 births to 92 females (NEAq right whale catalogue). In 1997, 73 of the known living adult females (N = 101) had been observed with calves. To identify age-specific differences in reproductive success among females, we examined the cumulative reproductive success of females alive in 1997 within three adult age classes: 9-12 years, 13-16 years, and > 17 years. Age classes were divided into intervals such that each interval accomodated a single calving event by each female (Knowlton *et al.* 1994). Using the 17-year reproductive histories of the 73 known reproductive females in the 1997 population, the number of calves born to each female within each interval was determined.

Paternity analysis

The proportion of males contributing to calf production was evaluated through paternity analysis using a combination of exclusionary principles and likelihood estimates (Marshall *et al.* 1998). When more than one male matched the genotype of the calf, a likelihood approach was used to rank males based on their probability of true paternity (CERVUS; Marshall *et al.* 1998). The likelihood of each male being the true father was determined using the ratio of the likelihood that the mother and putative ($L(H_1)$) father are the parents to the likelihood that the mother and a randomly chosen male are the parents ($L(H_0)$):

$$L(H_1)/L(H_0) = \frac{T(g_o/g_m,g_a)*P(g_m)*P(g_a)}{T(g_o/g_m)*P(g_m)*P(g_a)} ,$$

where g_o, g_m and, g_a are the genotypes of the offspring, mother, and alleged father, T is the Mendelian segregation probability, and P(g) is the frequency of a genotype in the population. The likelihood of paternity assigned to a given male is, therefore, influenced by how common the alleles shared between himself and the calf are in the overall population. Likelihood values calculated for each locus were combined and the natural logarithm of this combined likelihood ratio, or LOD score, was used to assign a probability of paternity to all males. Positive LOD scores indicate that the male being tested is more likely the father than a randomly selected male. The magnitude of the observed difference in LOD scores for the top two ranking males was then tested against

a critical ΔLOD value generated through computer simulations to assign a probability of paternity to the top ranking male. Marshall *et al.*(1998) suggest incorporating a mismatch error rate into paternity testing to account for possible mutation events and typing errors. As such, paternity analysis was assessed for 62 calves using exclusions based on a single mismatch (error rate = 0) and using likelihood assignments (3% error rate).

Mating System Analysis

To test the hypothesis that the mating system for *E. glacialis* involves male dominance polygyny, the observed distribution of males with single or multiple paternity assignments was compared with the distribution expected under random mating and several types of dominance polygyny. The probability of siring offspring followed the Poisson distribution and was set to be equal among males within age classes (*i.e.*, 11/100) but differed between age classes according to the number of years males were sexually mature. Under random mating, males that were mature throughout the 17-year period were expected to sire an average of 1.72 calves while those that were sexually mature for only one of the 17 years were only expected to sire 0.11 calves. The expected distributions of male mating success under dominance polygyny systems was estimated for cases wherein only 35%, 50%, or 60% of all males successfully sire offspring. Assuming an average of 100 adult males are present in the population in any given year, the average number of calves expected to be sired by breeding males over 17 years under the three levels of dominance polygyny would be 1.77, 1.24, and 1.03, respectively. Differences between the observed number of paternity assignments for males and the

expected number under random mating and three types of dominance polygyny were tested using the χ^2 test.

Kinship analysis

Coefficients of relatedness based on microsatellite genotypes were calculated for all pairs of individuals genotyped using the program Kinship 1.1.2 (Queller and Goodnight 1989; Goodnight *et al.* 1997). Relatedness coefficients (R_{xy}) reflect the probability that two alleles, one in each of two individuals (x and y), are identical-by-descent, where ($p_{i\text{-}j}$ - $P_{\text{-}i}$) is the difference between the allele frequency in individual 'i' (p) and the population (P), and the term (p_{ij} - $P_{\text{-}i}$) represents the difference between the allele frequency in the second individual ('j') relative to the frequency in the population:

$$R_{xy} = (p_{i\text{-}j} - P_{\text{-}i})/(p_{ij} - P_{\text{-}i})$$

(Queller and Goodnight 1989). To avoid potential bias in the calculation of population allele frequencies, the genotype of the individual being tested is excluded from the population frequency estimate ($P_{\text{-}i}$). Information from multiple microsatellite loci was combined by summing over alleles for all loci for each individual. Pairwise relatedness coefficients were calculated for all possible pairs of whales (209 x 209). R_{xy} coefficients were grouped for individuals of known relatedness (using the pedigree data) and the distribution of these observed values was tested against those generated through simulations based on the allele frequency data for the population (Queller and Goodnight

1989). R_{xy} values were generated for unrelated, half-siblings, and full siblings and these were tested against the frequency distributions for individuals of known relatedness from the population, including: parent-offspring, mating pairs, siblings, half-siblings, and unrelated individuals. Differences between the sets of distributions were tested using Monte Carlo simulations (10,000 simulations) using the software package REAP (Roff and Bentzen 1989). The relatedness coefficients for calves born within the same cohort year were also tested relative to simulated unrelated and half-siblings using the Monte Carlo approach.

Estimating the Effective Population Size

The N_e estimate for *E. glacialis* was made using the variance in reproductive fitness for males and females (*i.e.*, Inbreeding N_e; Kimura and Crow 1963). A second estimate was also made using the changes in allele frequencies observed across one generation (Variance N_e; Waples 1991). The observed mean (k) and variance (V_k) in reproductive success were calculated separately for males and females (*m* and *f*, respectively) based on the distribution of paternities among males and the reproductive histories of females. Mean (K) and variance (V_k) were combined for the two sexes as:

$$K = mk_m + (1-m)k_f$$

$$V_k = mV_m + (1-m)V_f + m(1-m)(k_m - k_f)^2 ,$$

where m and f are the proportion of males and females in the population. These values

were then used to calculate the inbreeding effective population size:

$$N_e = \frac{N_{t-2}\, K - 2}{K - 1 + V_k / K},$$

where N_{t-2} is the number of adults in the grandparental generation.

To estimate the variance N_e using changes in allele frequencies across generations (Waples 1991), the sighting records for individual *E. glacialis* were used to identify two age categories separated by one generation. Allele frequencies were calculated for those whales > 20 years old (N = 59) and those whales <10 years old (N = 55). The computation of this estimate is based on the changes in frequencies (x) of alleles (L) between the two sampled populations separated by 't' generations:

$$N_e = \frac{1}{L - 1} \sum_{i=1}^{L} \frac{(x_o - x_t)^2}{(x_o - x_t)/2} .$$

Results

Population Demography

Using the sighting-resighting histories for 388 identified whales from the NEAq right whale database, the numbers of males, females, and individuals of unidentified sex for the living 1997 population were estimated. The precise age is only known for whales that were identified in their year of their birth (N = 165), therefore, estimates of the number of individuals in each of several age classes were made by inferring the ages of the remaining whales using their sighting histories. The year of birth was assigned to individuals of unknown age as the year prior to their first sighting unless the first sighting involved a female with a calf. In the latter case, females were assumed to be at the average age of first parturition upon first sighting (nine years; Knowlton *et al.* 1994). Whales were classified into two subadult age classes (0-4 and 5-8 years) and three adult age classes (9-12, 13-16, and ≥17 years; Table 5-1). For each age class, individuals of unknown sex were assigned as males or females to maintain the 1:1 sex ratio observed in *E. glacialis* (Brown *et al.* 1994). The expected calf production by the 101 adult females in the population was estimated by assuming that all females over nine years of age produced calves at intervals of four years throughout the 17 year period (N = 425 calves). The number of expected births to females within each age class was then calculated as the product of the number of four-year calving intervals since the age of nine years and the number of females in an age class. The average (x) and variance (σ^2) in the number of calves produced per female were calculated from the reproductive histories of all known

reproductive females (living and dead) identified from 1980 to 1997 ($x = 1.70$ and $\sigma^2 = 2.6$; NEAq catalogue).

In 1997 the population consisted of an estimated 147 females, 101 of which were adults (67%) and 46 of which were subadults (33%). Only 73 of the 101 known adult females produced calves from 1980 to 1997 (Table 5-1). These breeding females produced 172 calves, which represent only 40% of the expected number for the adult females. The proportion of females in each age class that had produced a calf increased with age. Each age class, however, still only produced ~ 40% of the expected number of calves. The 9-12 year olds were expected to produce one calf each; only 58% of the females in this age group were known to have given birth. Although a larger proportion of females > 12 years old produced calves than among 9-12 year olds, only 65% and 81% of the females in the two oldest age classes had produced calves. Collectively, only 40 of the 73 known breeding females (55%) had produced more than one calf during this 17-year period (NEAq catalogue).

Paternity analysis

The 209 genetic profiles analysed included 100 of 165 photo-identified calves, 43 of the known reproductive females (N = 92), and 66 individuals of either sex with unknown birthing histories. The 62 mother-calf pairs evaluated in the paternity analysis included calves born from 1980 to 1997. Genetic profiles based on 9 microsatellite loci provided 97.6% exclusionary power for paternity analysis when samples were available for both mother and calf. To avoid excluding potential fathers, all males whose year of birth

Table 5-1. Number of females in each of four age classes for all female *E. glacialis* known to be alive in 1997.

Age class (years)	No. Known females	Estimated total No. females	Expected No. calves[1]	Observed No. calves[1] (% of expected)	No. Known breeding females	No. Calves/ female[2]
0-4	18	22	0	0	0	0
5-8	24	24	0	1	1	0.04
9-12	21	26	26	15 (58%)	15	0.57
13-16	18	23	46	19 (41%)	15	0.41
> 17	51	52	353	137 (39%)	42	2.6
	132	**147**	**425**	**172 (40%)**	**73**	

[1] The number of calves that a female could have produced is: zero for 1-4 and 5-8 year olds, one for the 9-12 year olds, and two for the 13-16 year olds. Given that 425 calves could have been born during the 17-year period, the remaining calves would have been produced by the > 17 year old category. [2] Average number of calves per living female.

was unknown were included in the analysis. This approach is expected to have resulted in the inclusion of juvenile males, thereby inflating the overall estimate of the proportion of adult males tested. The minimum and maximum estimates of the proportion of males included in the paternity analysis ranged across years from 60 to 75% with a within-year variance estimate of ~5%. The age of first reproduction is unknown for the males, however, one instance of a five-year old female producing a calf has been recorded (NEAq right whale catalogue). As such, two separate paternity tests were conducted. For the first analysis, the average age of first parturition for females was used as the minimum breeding age for males (nine years; Knowlton *et al.* 1994). In the second analysis, the potential for males to breed at a younger age was considered by including males of all ages in the analysis.

Neither the incorporation of a 3% error rate nor the inclusion of males less than nine years old had a strong effect on the identity of the top LOD-ranking males. Using a 0% error rate, 39 calves had 31 different males assigned the top-LOD score. Within these 39 matches, 24 fathers could be assigned with a match probability $\geq$ 80%. When males of all ages were included in the paternity analysis, two top-ranking LOD assignments changed, however, no additional calves were assigned a putative father. When paternity analysis was analysed using all males $\geq$ nine years and a 3% error rate, the identity of 10/39 top LOD score males changed but the proportion of calves with different top-ranking paternal matches was similar to those observed with an error rate of 0% (44/62 or 71%; Figure 5-1). Overall, the inclusion of a 3% error rate reduced the number of males that were assigned a match probability $\geq$ 80% but increased the number of calves with males

assigned LOD scores with positive values (N = 62).

Mating system analysis

The overall proportion of males assigned single or multiple paternities was similar for paternity analyses that used different error rates, therefore, the mating system was evaluated based on the results of the most conservative approach. Using the results from the analysis with a 0% error rate that included only those males over nine years of age, the proportion of males assigned 1, 2, 3, or 4 calves (N = 39) during the 17-year study period was tested against the expected distribution among males under random mating and dominance polygyny (Figures 5-1 and 5-2). Assuming the Poisson distribution, the average number of calves sired under each hypothetical mating strategy was calculated and used to represent the predicted distributions. The distribution of paternity assignments using a 0% and 3% error rate were consistent with that expected under random mating (Figure 5-1). In contrast, all three of the male dominance heirarchies tested (35%, 50%, and 60%; Figure 5-2) differed from the observed distribution ($p < 0.001$, $p < 0.05$ and $p < 0.05$, respectively). The relative proportions of males assigned single or multiple paternities to the 39 calves in our paternity analysis were used to create a distribution of paternity assignments for the 172 calves born from 1980 to 1997. The distribution generated from the observed data was similar to that expected through random mating although a larger proportion of males were assigned one paternity.

Using the average number of male calves born each year (N = 5), the predicted number (N = 168) and age distribution of adult males alive from 1980 to 1997 was

generated. Under random mating, 60% of these adult males would be expected to sire calves. To determine the effect that incomplete sampling of breeding males might have on the expected distribution of paternities, we excluded 25% and 50% of actual breeding males to simulate incomplete sampling under 40% dominance polygyny (Figure 5-3). The effect of excluding actual breeders from the pool of sampled males increased the number of males with no paternity assignments and decreased the number of males assigned one paternity, making the paternity data appear more similar to a 35% dominance hierarchy ($p > 0.05$). Given that the data suggest that mating is random in this species, estimates of the mean and variance in male reproductive success were made using the expected distribution of male reproductive success for 172 births ($x = 1.0$ and $\sigma^2 = 1.2$).

Kinship Analysis

Relatedness simulations based on the observed allele frequencies in the population were run to establish the distribution of R_{xy} values for unrelated, half-sibling, and parent-offspring relationships. The mean and standard deviations for these relationships were made based on 1000 simulations (0.0007 ± 0.24, 0.24 ± 0.23, and 0.49 ± 0.21, respectively) and were similar to the R_{xy} values observed among individuals of known relatedness. However, small sample sizes prevented statistical analysis between some relationships. R_{xy} values for calves born in the same cohort year were assessed to determine if they were being sired by a limited number of males by comparing the observed values among calves to the simulated values for half siblings and unrelateds. R_{xy}

values for five of the ten cohort years for which more than five calves had been genotyped could be discriminated from the unrelated and half-sibling distributions generated through the simulations (Figure 5-4). For the five cohorts, the distributions of R_{xy} values were consistent with expectations among unrelated individuals ($p > 0.05$) but differed from that of half-siblings ($p < 0.001$).

Inbreeding analysis

The paternity data used to examine levels of inbreeding among mating pairs was restricted to only the 24 paternity assignments with a probability of paternity $\geq$ 80%. When the relationship among breeding pairs was compared, the R_{xy} values had a mean well below that among half siblings (-0.035 $\pm$ 0.28 versus 0.24 $\pm$ 0.23). No one cohort year had a large proportion of calves included in the paternity analysis, however, the proportion of calves genotyped within cohort years was high (71% $\pm$ 13%).

Estimating the Effective Population Size

The mean and variance in male reproductive success estimated under random mating for the production of 172 calves ($x = 1.0$ and $\sigma^2 = 1.2$) and that known for the 101 living adult females in the 1997 population ($x = 1.7$ and $\sigma^2 = 2.6$) were used to estimate the inbreeding N_e at 154. In contrast, the N_e estimate based solely on changes in observed allele frequencies across one generation placed the N_e lower than the inbreeding estimate (Variance $N_e = 103$). A third estimate based on the number of breeding females ($N_f = 72$) and males (assuming random mating, $N_m = 60$) in the population was also used. For this

estimate,

$$N_e = \frac{4 N_m N_f}{N_m + N_f}$$

(see Nei 1987) the N_e was intermediate between the variance and inbreeding estimates ($N_e = 132$).

Figure 5-1. Distribution of male reproductive success from paternity analysis. Results of paternity analysis with 0% and 3% error rates showing the number of males assigned 1, 2, 3, or 4 paternities are contrasted to predicted distributions under random mating.

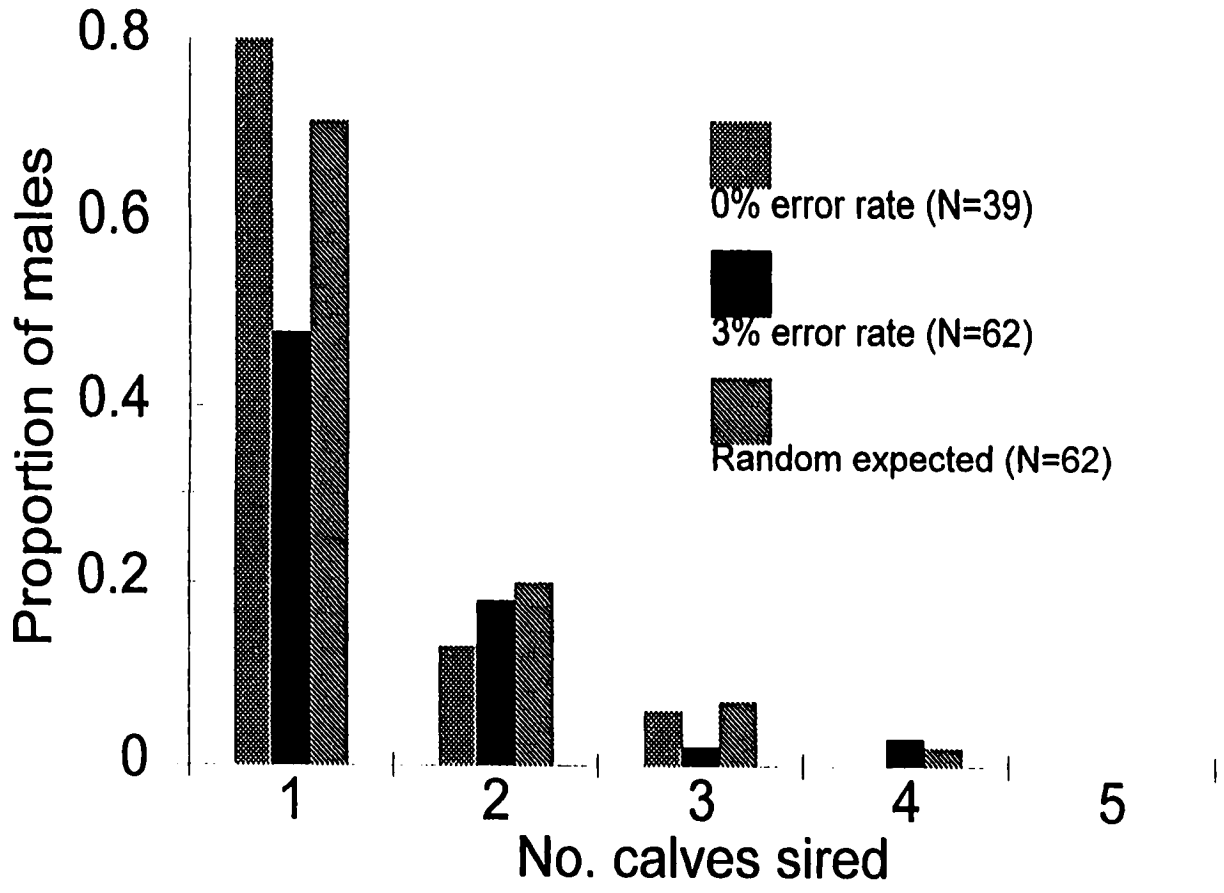

0.8
0.6
0.4
0.2
0
Proportion of males
0% error rate (N=39)
3% error rate (N=62)
Random expected (N=62)
1
2
3
4
5
No. calves sired

Figure 5-2. Expected distribution of male reproductive success under different dominance polygyny mating strategies. The expected distributions for dominance heirarchies in which only 35%, 50%, or 60% of all males breed are shown for 62 births.

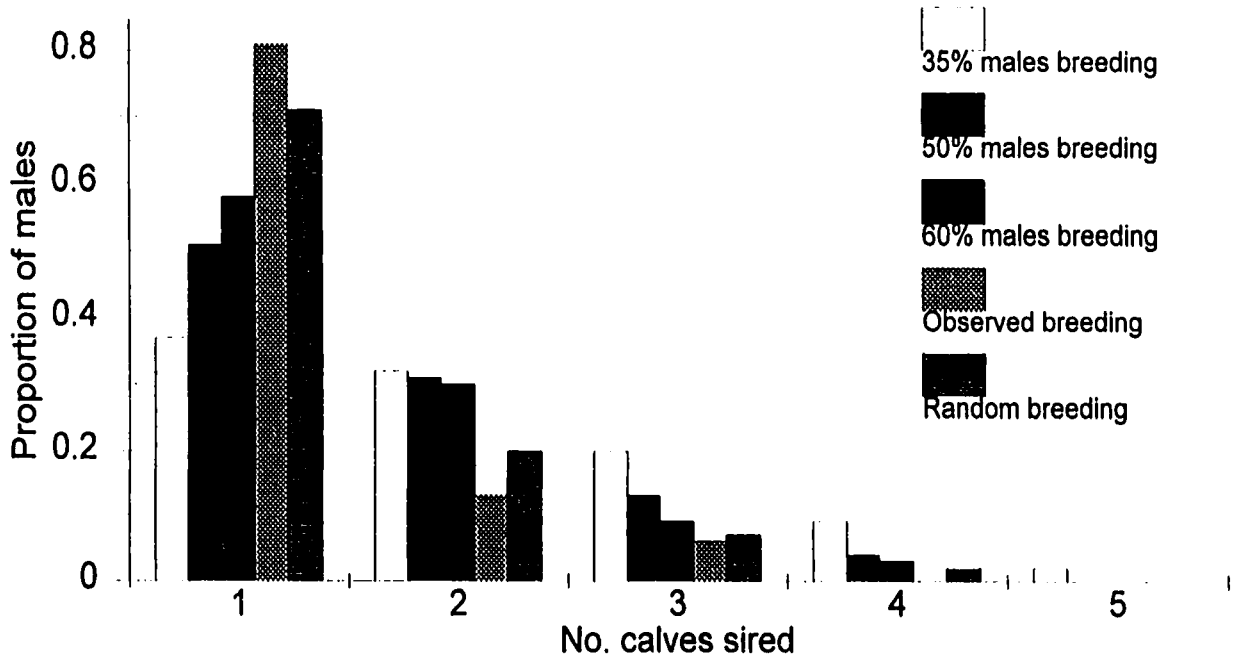

0.8
0.6
0.4
0.2
0
Proportion of males
1
2
3
4
5
No. calves sired
35% males breeding
50% males breeding
60% males breeding
Observed breeding
Random breeding

Figure 5-3. Distribution of male reproductive success under a 40% dominance hierarchy with incomplete sampling. Simulations of incomplete samplings of breeding males (25% and 50% of breeders unsampled) are contrasted to that expected from a 30% dominance hierarchy and random mating.

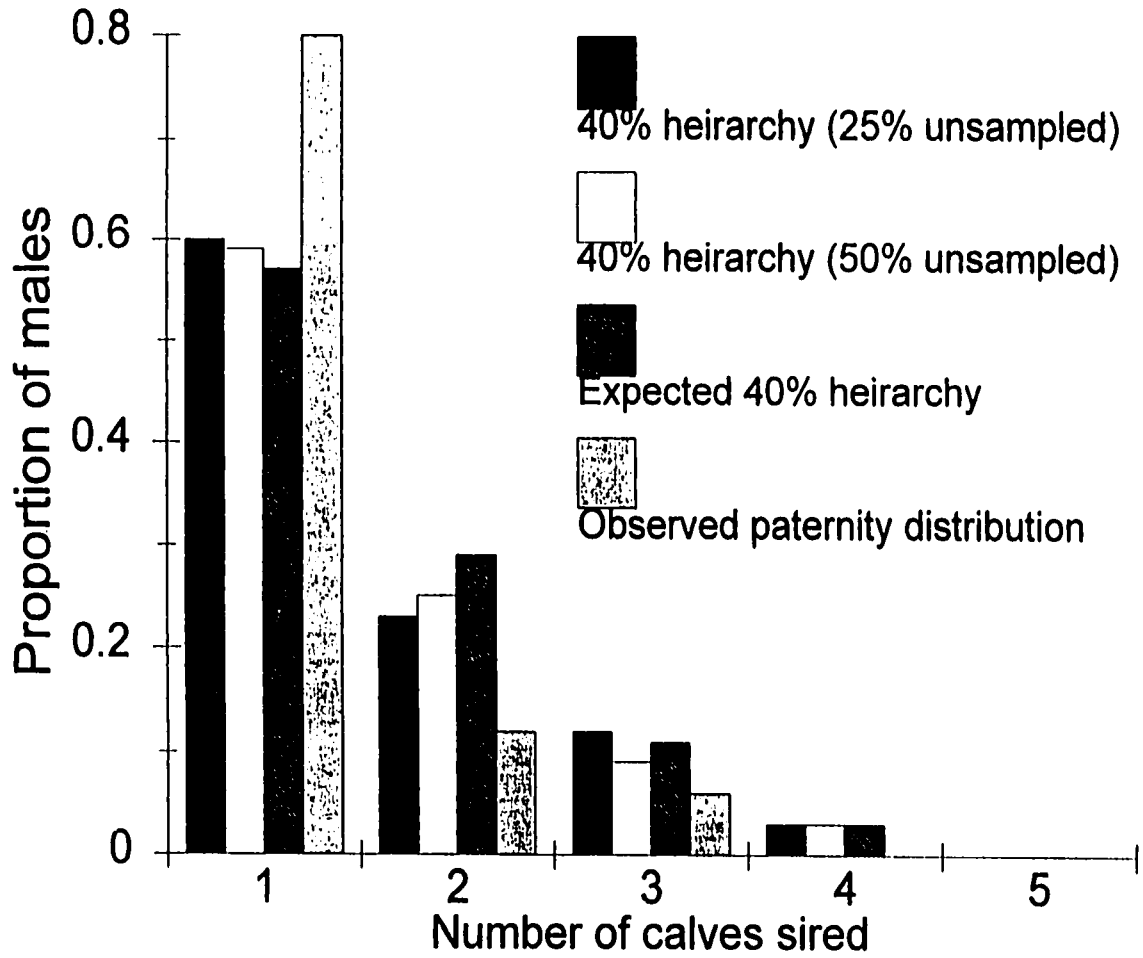
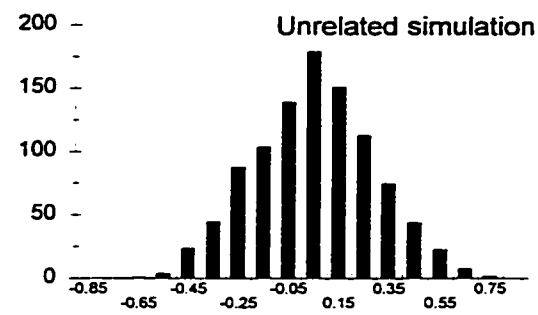
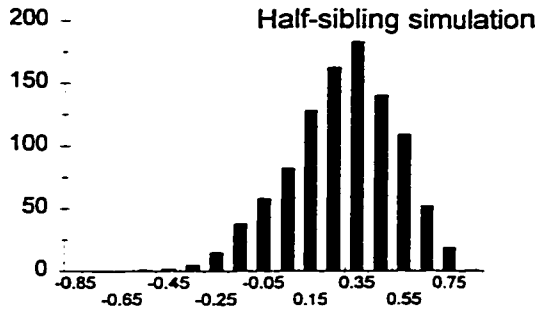
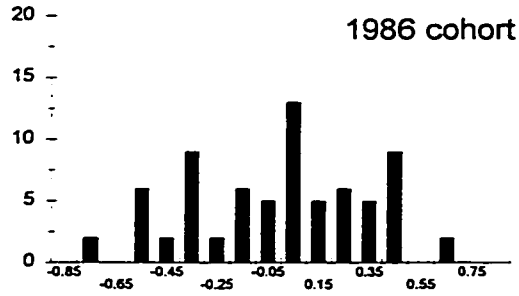
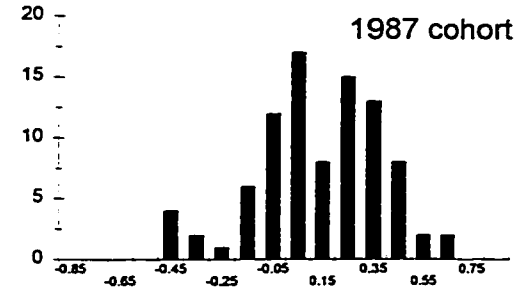

0.8
0.6
0.4
0.2
0
Proportion of males
40% heirarchy (25% unsampled)
40% heirarchy (50% unsampled)
Expected 40% heirarchy
Observed paternity distribution
1
2
3
4
5
Number of calves sired

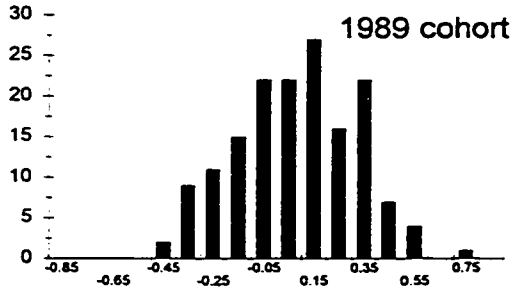
Figure 5-4. Frequency distribution of relatedness coefficients (R_{xy}) for calves born in the same cohort year. R_{xy} values are shown for the six cohort years tested against expected values among pairs of unrelateds and half-siblings.

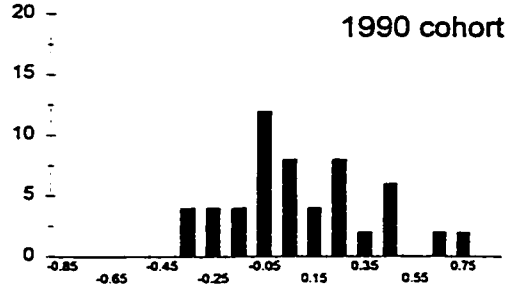

Unrelated simulation
200
150
100
50
0
-0.85
-0.65
-0.45
-0.25
-0.05
0.15
0.35
0.55
0.75

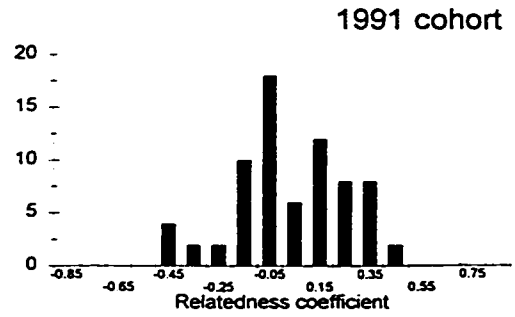

Half-sibling simulation
200
150
100
50
0
-0.85
-0.65
-0.45
-0.25
-0.05
0.15
0.35
0.55
0.75

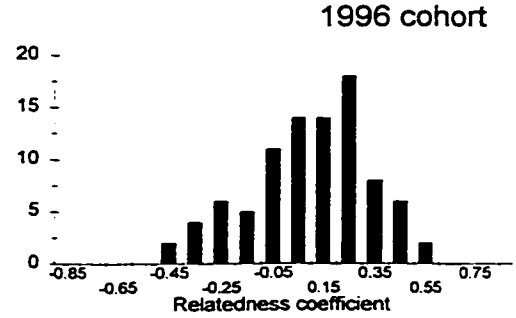

1986 cohort
20
15
10
5
0
-0.85
-0.65
-0.45
-0.25
-0.05
0.15
0.35
0.55
0.75


1987 cohort
20
15
10
5
0
-0.85
-0.65
-0.45
-0.25
-0.05
0.15
0.35
0.55
0.75


1989 cohort
30
25
20
15
10
5
0
-0.85
-0.65
-0.45
-0.25
-0.05
0.15
0.35
0.55
0.75


1990 cohort
20
15
10
5
0
-0.85
-0.65
-0.45
-0.25
-0.05
0.15
0.35
0.55
0.75


1991 cohort
20
15
10
5
0
-0.85
-0.65
-0.45
-0.25
-0.05
0.15
0.35
0.55
0.75
Relatedness coefficient


1996 cohort
20
15
10
5
0
-0.85
-0.65
-0.45
-0.25
-0.05
0.15
0.35
0.55
0.75
Relatedness coefficient

Discussion

The mating system of the North Atlantic right whale is a polygynous one that maximizes the number of successful breeding males, thereby increasing the N_e and slowing the rate at which genetic variability is lost (Nei 1987). This finding contradicts behavioural observations which suggested that males compete for access to females and that older males are more successful in obtaining and maintaining positions favourable for copulation (Kraus 1991). These observations suggested that a limited number of males may breed successfully in a given year, however, there is no evidence to support this interpretation. Calves born within the same year were not more closely related than unrelated individuals and both the relatedness and paternity data suggest that an assortment of different males are siring calves born both within and across years.

If mating within the population were completely random, the variance N_e estimate would be expected to be similar to the inbreeding N_e (Lande and Barrowclough 1987). The observed difference between these estimates, although small (N_e = 103 and 154, respectively), suggests that mating may not occur randomly within the overall population. This interpretation is consistent with the genetic structuring recently identified between two group of whales whose mothers utilize different nursery grounds (Malik *et al.* 1999; Waldick *et al.* In Prep.). Although gene flow occurs between these two groups, sufficient structuring exists to maintain different allele frequencies in the two populations (Waldick *et al.* In Prep.). If the population is structured and mating does not occur randomly within a single panmictic population, the N_e for the overall population would be expected to be closer to the smaller variance estimate of 103. The difference between the two N_e

estimates may correspond with non-random mating within the overall population (Waldick *et al.* In Prep.). Nevertheless, the promiscuous nature of the mating system (lottery polygyny) should maximize the N_e within each subpopulation. In fact, the ratio of N_e to the census population size for this species is within the range of those reported for other, large-bodied mammals (Frankham 1995).

The absence of evidence of matings between closely-related individuals, such as might be expected under dominance polygyny, suggests that inbreeding depression may currently be having limited effect on this population. Given that the North Atlantic right whale population appears to have maintained a small, but relatively stable effective population size for over 100 years (Waldick *et al.* In Prep), it is unlikely that the low calf production by females can be attributed to a recent increase in the incidence of mating among relatives. The progressive loss of genetic variability throughout 700 years of commercial whaling may, however, have affected female fecundity through the loss of heterosis (see Waldick *et al.* In Prep.). Low variability at loci involved in reproduction, for example, has been linked to increased incidence of spontaneous abortion of fetuses (e.g., Human Leukocyte Antigen complex; Ober *et al.* 1998).

The low observed reproductive success among females does not appear to be a consequence of infertility, such as might be expected if inbreeding depression were affecting the population. In fact, the majority of the females (~74%) have at least one calf by the time they are 20 to 30 years old. The main cause for the small number of calves produced by this population is associated with the failure of many females (45%) to produce multiple calves during their lifetime. If genetic variability was lost from this

species more than 200 years ago, low calf production by North Atlantic right whales may have been characteristic for this population throughout this time. However, contemporary influences may also be limiting reproduction by this species. Resource shortages and disturbances within feeding grounds, or other areas used for reproduction, may be interferring with mating or feeding activities (Kraus 1990; Caswell *et al.* 1999). Relatively high mortality has been observed among first-time mothers and may indicate that human-related mortalities also affect calf production (NEAq catalogue).

Our analysis of the mating system and relatedness structure in the endangered North Atlantic right whale provides no evidence to suggest that inbreeding is occurring in this population. Rather, inbreeding depression is likely to have occurred more historically, in association with the bottleneck events of the 15th and 18th centuries (Waldick *et al.* In Prep.). The recent decline in the growth rate in the North Atlantic right whale is more likely the result of the combined effects of low genetic variability at genes involved in reproduction and reduced survivorship. Although population bottlenecks due to intensive whaling pressures over a period of 700 years have resulted in the loss of genetic variability in the North Atlantic right whale (Schaeff *et al.* 1997; Waldick *et al.* In Prep.), there is no evidence of infertility among females and the mating system is one which maximizes the retention of genetic variability. This population has shown itself capable of persisting for 100-200 years at a small population size. However, its resiliance to additional stresses may be tenuous. Given that the existing model of population growth for *E. glacialis* (based on current reproductive and survivorship data) predicts that the population will go extinct within the next two centuries (Caswell *et al.* 1999), it is

imperative that efforts be made to identify and mitigate those influences that may be exacerbating the low calf production by this species.

Acknowledgements

This research was supported by an N.S.E.R.C. Scholarship to RCW, N.S.E.R.C. Research funding awarded to BNW, and funding to SK and MB from the National Marine Fisheries Service (USA). Thanks to Jim Quinn and Lisle Gibbs for their recommendations regarding data analysis.

Literature cited

Amos W, Barrett J, Dover GA (1991) Breeding behaviour of pilot whales revealed by DNA fingerprinting. Heredity, 67, 49-55.

Brown MW, Kraus SD, Gaskin DE, White BN (1994) Sexual composition and analysis of reproductive females in the North Atlantic right whale, *Eubalaena glacialis*, population. Marine Mammal Science, 10, 253-265.

Bruford MW, Wayne RK (1993) Microsatellites and their application to population genetic studies. Current Opinions in Genetics and Development, 3, 937-943.

Burnell SR (1998) Aspects of the reproductive biology and behavioural ecology of right whales off Australia. Proceedings of the International Whaling Commission. Cambridge U.K. Doc. SC/M98/RW19.

Caswell H, Fujiwara M, Brault S (1999) Declining survival probability threatens the North Atlantic right whale. Proceedings of the National Academy of Science, USA, 96, 3308-3313.

Chakraborty RM, Meagher TR, Smouse PE (1988) Parentage analysis with genetic markers in natural populations. I. The expected proportion of offspring with unambiguous

paternity. Genetics, 118, 527-536.

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics, 18, 237-268.

Clapham PJ, Palsbøll P (1997) Molecular analysis of paternity shows promiscuous mating in female humpback whales (*Megaptera novaeangliae*, Borowski). Proceedings of the National Academy of Science USA, 93, 95-98.

Double MC, Cockburn A, Barry SC, Smouse PE (1997) Exclusion probabilities for single-locus paternity analysis when related males compete for matings. Molecular Ecology, 6, 1155-1166.

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. Genetical Research, Cambridge, 66, 95-107.

Frankham R (1998) Inbreeding and extinction: Island populations. Conservation Biology, 12, 665-675.

Goodnight K, Queller DC, Poznansky T (1997) Kinship. Version 1.1.2. http://www.bioc.rince.edu/~kfg/GSoft.html.

Keane B, Dittus WPJ, Melnick DJ (1997) Paternity assignment in wild groups of toque macaques *Macaca sinica* at Polonnaruwa, Sri Lanka using molecular markers. Molecular Ecology, 6, 267-282.

Kimura M, Crow JF (1963) The measurement of effective population number. Evolution, 17, 279-288.

Knowlton AR, Kraus SD, Kenney RD (1994) Reproduction in North Atlantic right whales (*Eubalaena glacialis*). Canadian Journal of Zoology, 72, 1297-1305.

Kraus SD (1990) Rates and potential causes of mortality in North Atlantic right whales (*Eubalaena glacialis*). Marine Mammal Science, 6, 278-291.

Kraus SD (1991) mating strategies in the North Atlantic right whale (*Eubalaena glacialis*). MSc. thesis, University of Massachusetts, Boston.

Lacy RC, Alaks G, Walsh A (1996) Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. Evolution, 50, 2187-2200.

Lande R, Barrowclough GF (1987) Effective population size, genetic variation, and their use in population management. Viable Populations for Conservation. Ed. Soule ME. Cambridge University Press, Cambridge. Pp. 87-124.

Malik S, Brown MW, Kraus SD, White BN (1999) Assessment of mitochondrial DNA structuring and nursery use in the North Atlantic right whale (*Eubalaena glacialis*). Canadian Journal of Zoology.

Malik S, Brown MW, Kraus SD, White BN (In Press) Analysis of mitochondrial DNA diversity within and between North and South Atlantic right whales. Marine Mammal Science.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology, 7, 639-655.

Nei M (1987) Molecular Evolutionary Genetics Columbia University Press, New York.

Nunney L, Elam DR (1994) Estimating the effective population size of conserved populations. Conservation Biology, 8, 175-184.

Ober C, Hyslop T, Elias S, Weitkamp LR, Hauck WW (1998) Human leukocyte antigen matching and fetal loss: results of a 10 year prospective study. Human Reproduction, 13, 33-38.

Palsbøll PJ, Allen J, Berubé M, Clapham PJ, Feddersen TP, Hammond P, Hudson RR,

Jørgensen H, Katona S, Larsen AH, Larsen F, Lien J, Mattila DK, Sigurjønsson J, Sears R, Smith T, Sponer R, Stevick PT, Øien N (1997) Genetic tagging of humpback whales. Nature, 388, 676-679.

Parker PG, Snow AA, Schug MD, Booton GC, Fuerst PA (1998) What molecules can tell us about populations: choosing and using a molecular marker. Ecology, 79, 361-382.

Payne R, Rowntree V, Perkins JS, Cooke JG, Lankester K (1990) Population size, trends, and reproductive parameters of right whale (*Eubalaena australis*) off Peninsula Valdes, Argentina. Report of the International Whaling Commission, special issue, 12, 271-278.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution, 43, 258-275.

Queller DC, Strassmann JE, Hughes CR (1993) Microsatellites and kinship. Trends in Ecology and Evolution, 8, 285-288.

Ralls K, Brugger K, Ballou JD (1979) Inbreeding and juvenile mortality in small populations of ungulates. Science, 206,1101-1103.

Richard KR, Dillon MC, Whitehead H, Wright JM (1996) Patterns of kinship in groups of free-living sperm (*Physeter macrocephalus*) revealed by multiple molecular genetic

analysis. Proceedings of the National Academy of Science, USA, 93, 8792-8795.

Rockwell RF, Barrowclough GF (1995) Effective population size and lifetime reproductive success. Conservation Biology, 9, 1225-1233.

Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem with small sample sizes. Molecular Biology and Evolution, 6, 539-545.

Schaeff CM (1993) A genetic analysis of right whales (*Eubalaena*) in the Atlantic ocean. PhD. Thesis, Queen's University, Kingston.

Schaeff CM, Kraus SD, Brown MW, White BN (1993) Assessment of the population structure of western North Atlantic right whales (*Eubalaena glacialis*) based on sighting and mtDNA data. Canadian Journal of Zoology, 71, 339-345.

Schaeff CM, Kraus SD, Brown MW, Perkins JS, Payne R, White BN (1997) Comparison of North and South Atlantic right whale (*Eubalaena*) genetic variability using DNA fingerprinting. Canadian Journal of Zoology, 75, 1073-1080.

Schlotterer C, Amos B, Tautz D (1991) Conservation of polymorphic simple sequence loci in cetacean species. Nature, 354, 63-65.

Sokal RR, Rohlf FJ (1981) Biometry. 2nd ed. Freeman and Company, New York.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research, 17, 6463-6471.

Waldick RC, Brown MW, Kraus SD, Wilson PJ, White BN (In Prep) Assessment of the effects of historic bottlenecks on the microsatellite DNA variation in the western North Atlantic right whale (*Eubalaena glacialis*) relative to that in a related species (*Eubalaena australis*).

Waldick RC, Brown MW, Kraus SD, White BN (In Press) Characterisation and Isolation of microsatellite loci from the endangered North Atlantic right whale. Molecular Ecology.

Waples RS (1991) Genetic methods for estimating the effective size of cetacean populations. Report of the International Whaling Commission, Special Issue 13, 279-301

Chapter six

General Discussion

Whales represent a fascinating group of vertebrates which inhabit aquatic environments. However, because of the logistic difficulties of studying large organisms in marine habitats, many fundamental aspects of their behavior and ecology remain poorly-understood. Much of the difficulty in studies of cetaceans is associated with difficulties in indentifying individuals. By using genetic markers to develop individual-specific genetic profiles for North Atlantic right whales, my work has provided new insights into important aspects of the behaviour of this large whale, including some that may also be helpful in conserving this animal. In particular, I have been able to 1) document previously-unrecognized genetic structuring within the population, 2) provide new insights into the role that 700 years of commercial whaling had on the genetic composition of this species, and 3) identify the nature of the mating system. I briefly expand on these contributions below.

The small size of this population combined with the collection of photographic records over a 19 year period have enabled most of the whales in the population to be individually identified. Using these records, the location and timing of use of important feeding, calving, and nursery habitats have been identified and behavioural differences in the use of these habitats have been noted among subsets of the population. These known habitat areas are now routinely monitored and measures have been taken to protect whales while they are within these waters. However, because some members of this population use habitat areas other than those that have been identified along the east coast of North America, not all members of this population are afforded protection by these efforts. Given the tenuous reproductive success of this population, the identification and

protection of all areas used for reproductive activities are critically important. Behavioural differences in nursing ground use between maternal lineages have confirmed the existence of an alternate nursery ground (Schaeff 1994; Schaeff *et al.* 1993; Malik *et al.* 1999). By identifying genetic structuring between these two groups of whales (BoF and Non-BoF) my study shows that the two groups are also reproductively isolated, which suggests the existence of two separate mating grounds. Given the low calf production by this population, it is critically important to identify these unknown habitats so that any disturbances that may be interfering with reproduction may be mitigated.

The reproductive histories of 73 females over a 17-year period showed that most females are fertile but that relatively few (45%) have successfully produced multiple calves during this time. The reasons for the low calf production by females include extrinsic influences such as resource limitations, disturbance, or habitat loss, and intrinsic limitations associated with inbreeding or low genetic variability. Given that the historic population size for North Atlantic right whales was in the thousands, it is unlikely that resource limitations are currently playing a major role in the low fecundity of females in this small population. However, disturbances at the mating, calving, or nursery grounds may interfere with reproduction or reduce survivorship, both of which would be expected to have a deterimental effect on reproduction. Once identified, the effects of these extrinsic influences may be mitigated. If, however, intrinsic influences are affecting female reproductive fitness, the current rate of calf production by females may represent the maximum for this species. If low calf production is an intrinsic characteristic of this species, the population growth rate may only be increased by directing conservation

efforts at reducing the mortality rate for this species.

Through the analysis of the heterozygosity in the population and bottleneck analysis, the loss of genetic variability was identified as having occurred prior to the 19th century. Given the antiquity of this loss, the observation that most females are fertile, and the absence of evidence of inbreeding in the current population, it is unlikely that inbreeding depression is having a substantial influence on the low calf production in this population. Rather, it may be the low levels of genetic variability that have limited reproduction through the loss of heterosis (e.g., see Ober *et al.* 1998). The low calf production by females would, therefore, be characteristic of this population for over one hundred years. As such, the recent decline in the growth rate of this population would be the result of a recent increase in mortality which cannot be compensated for by the intrinsically low calf production by females.

The status of the endangered North Atlantic right whale may not, however, be as tenuous as these findings initially suggest. The finding that the population consists of two reproductively isolated groups, when combined with the observation that most of the new sightings tested represent previously unidentified whales, suggests that the North Atlantic right whale population is larger than was previously thought. Some of the unknown whales are expected to be members of the Non-BoF group which are sighted less often than the BoF whales because they occupy habitat areas other than those that are routinely monitored by the Right Whale Consortium group. Through maternity and paternity analyses, our genetic data enabled new sightings to be discriminated as previously unknown whales or known births that had not been photo-identified. Once identified as

such, the membership of these whales to the BoF or Non-BoF groups may be assigned based on their Individual index value. Using this approach, the census population estimate may be refined even in the absence of high quality photographs. Molecular-genetic profiles provide a powerful dataset for augmenting field-based natural history studies, particularly for secretive species like the cetaceans.

Recommendations for future research

1) Given the recent decline in the growth rate of this species, the identification and protection of all areas used for reproductive activities must become a management priority. Satellite tagging of adult males identified as belonging to the BoF and Non-BoF groups will be necessary to track whales to their respective mating grounds. In addition, it is critically important that the location of the second nursery ground be identified. Intra-annual differences in calf production between the two groups may reflect different stresses that are affecting the whales in their mating or nursery habitats. In order to mitigate any anthropogenic influences that may be detrimentally affecting these populations, these habitats must be protected.

2) Individual genetic profiles should be expanded to include additional microsatellite loci and functional genes (e.g., Major Histocompatability Complex) so that whales with unknown birthing histories may be assigned to the BoF or Non-BoF groups. These profiles would also increase the ability to assign paternities and maternities, thereby facilitating the refinement of both the census and effective population sizes.

3) Genetic loci that have been shown to be important for growth and reproduction (e.g., Major Histocompatability Complex, Insulin-like Growth Factor etc.) should be examined and examined relative to female reproductive success and inbreeding among mating pairs to determine if the loss of genetic variability is limiting calf production. If most calves are heterozygous at these loci, and the locus is out of HWE, this would support the hypothesis that low genetic variability (loss of heterosis) is limiting calf production by females.

Literature Cited

Malik S, Brown MW, Kraus SD, White BN (1999) Assessment of mitochondrial DNA structuring and nursery use in the North Atlantic right whale (*Eubalaena glacialis*). Canadian Journal of Zoology.

Ober C, Hyslop T, Elias S, Weitkamp LR, Hauck WW (1998) Human leukocyte antigen matching and fetal loss: results of a 10 year prospective study. Human Reproduction, 13, 33-38.

Schaeff CM (1993) A genetic analysis of right whales (*Eubalaena*) in the Atlantic ocean. PhD. Thesis, Queen's University, Kingston.

Schaeff CM, Kraus SD, Brown MW, White BN (1993) Assessment of the population structure of western North Atlantic right whales (*Eubalaena glacialis*) based on sighting and mtDNA data. Canadian Journal of Zoology, 71, 339-345.

Appendix one

Microsatellite nucleotide sequences from the North Atlantic right whale. Sequences that were found to be polymorphic in North and/or South Atlantic right whales are presented in this appendix. Primer sites/sequences are highlighted in bold and underlined. Clonal sequences are indicated for each known sample.

(I) RW18 (CLONE 4-2, SAMPLE 18)

CTTGCATGCGCCNTTCNACTCTAGAGGATCCCCCTCTGGAGCACGGAATGACT

CC**AGAGGGAAGCAAACTGGA**AAAGGAGGATGGGGAGAGAGGGAGGGCGTG

TATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTTGGCAGGGGGG

GTGGTGGTTGGAGAANAAAGACGGGAAGTAAGGATCAACAAAGAGCCTGGG

ATGCTGGGAAATGGCATATTTAAG**GGGGTGTCTGGCANCCTTC**TTAAGGTG

GAACCTTTTCAGCAGGGTTTTCTCGGTTGCAGGGGTACCGAGCTCNAATTCGT

AATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTA

(II) RW26 (CLONE 4-9, SAMPLE 25)

GTCCATCCATATTACTGCAAATGGCATTACTTCATTCTTTTTTACAGCTGAGTA

ATATTCTANTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATACATGCACC

ACATCTTCTTTACAGGATTTCCTTTTTTTTTTTTAACA**GCTTCATTGAGGTAT**

AACTG

(III) RW31 (CLONE 4-15, SAMPLE 31)

TGCGCNCCNTCNACTCTACAGGACGCCCAACAAATTTCAAATGATCATT**TAT**

TCATGGAGTGCTTTGGAATTAGAGTAGTATATATCATTTGAGTGGAGTGTGT

GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTACATGGCAATAAATAT

AACTACCACACTGGACTCTAGGTGGCTATCAGCACAGGCTTCAACCATTGTAT

TCTGGCAGGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCTG

TGTGAAATTGTTATCCGCTCACAATTCCA

(IV) RW34 (CLONE 4-18 SAMPLE34)

ACTCAAG**CCCCATAACGGCGCAT**AACACACACACACACACACACACACACA

CACACACACACACACACACACACACAGNACAGTNGNNCGTGCCCCTCAATTATA

TAAAGTATCA**GGTTCTGGCTCCCGA**AACANAGNATTCATCTGTTANCTGAA

AAATATGGANTTATCTGG

(V) RW45 (CLONE 4-5, SAMPLE 21)

CCGTATT**GCATCACAGCAAAAGGTCTTTCATTGCTGCC**GCTGCTTTGTGTG

TGTGTGTGTGTGTGTGTGTGTGTGTATGTATGAGAGAGAGAGAGAGAGAGAA

TGTGTAAACAGATCTCTCTGTGGAATAGTTTTGAAG**TAAGGCTTTCTGATTT**

CCGTGTTTCTAGTTGCTTATGTGCAACTTGCTTTTCTGTATCTCAGANGAAAC

AGCCAGTCTGGCCATGGTCTGATTTTGTCTGTCCATGCAACTAAACGTTACTA

CCTCCACAAACTTCCTTAGGAAGGAATTCCTGAATTTCCTAAAA**GGGGAATT**

GTAAGTGATGTTTGAGGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGC

TGTTTCCTGTGTTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGC

CGGAAGCATAAAGTTGTTAAAGCCTGGGGTGCCTAATGAGTGAG

(VI) RW48 (CLONE 4-8, SAMPLE 24)

AGTTTTGTGTGATTTTAAAAATAAAGACTGTGTATTCTTTTCTATAGCATTAAC

ATAATTTTCTTAAAAAGGAAGAAGAAATTAGTGTCTTGAGAAGTAATTGAAA

G**CCAATGACTTTTCCCTGTA**GATTTCATAAGGGTTTGTGTGTGTGTGTGTGT

GTGTGTGTGTGTGTGTGTGTGTGTGTGTGGTGGGGACAGCANTGTTTCAGTGC

ATTTGTTCC**CAGGANACACTGCGGTATC**AGAGTTCAATGGAATGAGGGGGG

TACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGT

TATCCGCTCACAAT

(VII) RW417 (CLONE 4-17, SAMPLE 33)

CTACAGGACNCCCN**TGTATCCTGCAACC**TTGCTGAACTCGTGTGTGTGTGTG

TGTGTGTGTGTGTGTGTGTGTGTGTATTTTTTTTAGTGGATTCC**TTAGGATTTT**

CTTTATGCAAGGTCATGTCATCTGTGAATAGAGATAGTTTTATTTTTTCCTTT

CCAATCTATTTGCTTTTTATTTCTTTTTCTTGCCTGGCGCTAGAACCTGCAGTA

AGTTATTTACTTAATACTGAATACAAGTGGCAGGAACAGACATCCTTATCTTG

TTCCTAATCTTAGGGCAAAAGCGTTCAGT

(VIII) RW219 (CLONE 2-19)

ATC**AGTTCCATAGGGCTGCTCAC**AGCATAGCTTCTTAATTCTCACACACACA

CACACACACACACATTGG**GATTGAACCCAAAATGGAA**ATCAGTGATTTGTA

GACAACCTAAACTTGTCATAAGTGAT

(IX) EGL2-12 (CLONE 2-12)

ATC TCA CTT CTC CAA AAT A**GA CCA GAA ATA AAT CC**A TTT TAT TAA ATA

TGT GAC ACT TTT CCG CTT TAG GAT GGC CCA CTA CTC TGTG TGTG TGTT

TGTG TGTG TGTG TGCA CACG CA TGTG TATG TGTG TGTC CTTT TATA TGTT

CT**TG GTTA GGAT GGAA GCTT TT**TT TGGG GGTA AGTT TTAA ACTA TATA

ATTG ACTT AAGA GCAA GTGT AATT TTTC ACTG AAAC TTA TTC TTGA

GTCC TAAT CAGA T

(X) RW217 (CLONE 217)

ATC TGG CAT TTG TTT TAA AAT AAT CCT GTT AAT GAG TGT GTG TGT

GTG TGT GTG TGT GTG TCT AGT GAC TAG AGG TAG AAA TGAAAC AAA ACT

GGT CAC ATG CTG ACA ATG ATG AAG CTG GGT GAT GTG AGA GGG TAC ATT

GGG TTT ATT ACA TTA TTC TTT CTGGTT TTG TAA TAT ACA CTG TCA TAA

AAA ATA AAG TTT AAA AAA TGT ATA AGT CAT TAA TTT CTA TAT CGT GAT;

(XI) RW410 (CLONE 4-10 SAMPLE 26)

ATTTCACTTGTATGACAATCTCTAGGTCCATCCATATTACTGCAA**ATGGCATT**

ACTTCATTCTTTTTTACAGCTGAGTAATATTCTAGTGTGTGTGTGTGTGTGTG

TGTGTGTGTGTGTGTATACATGCACCACATCTTCTTTACAGGATTTCCTTTTTT

TTTTTTAACAGCTTCATTGAGGTATAACTGATACAGAGTAGAAGTTGTACATG

TTTGATATA**CACAATTTGGTAAGTTTGGC**ATGTGCATGTTATGAGCTTGACA

TGTGCATTATCAACCCATGATGGGTACCGAGCTCGAATTCGTAATCATGGTCA

TAGCTGTTTCCTGTGTTGAAATTGTTATCCGCTCACAATTCCACACAACATAC

GAGCCGGAAGCATAAAGTTGTAAAGC

Appendix two

Microsatellite nucleotide sequences from the North Atlantic right whale. The following Appendix includes nucleotide sequences that were found to contain microsatellite repeats but were not tested in right whales. Clonal sequences are indicated for each known sample.

(I) RW166 (CLONE 4-26 SAMPLE 6) SHOULD BE RE-TRIED WITH NEW REVERSE PRIMER

ACCAGCAAAACCAGCATCACTCTATCAATTAATGTGTGTGTGTGTGTGTGTG

TGTGTGTGTGTGTCTTGTGATGGGATAA**CCANTCANTAGGGG**TTTTAGTTAT

GCTGAATTGATTTTTTAATC

(II) RW01 (SAMPLE 01 CLONE 4-21) SHOULD BE RE-TRIED WITH NEW PRIMERS

CTTGCAAATTACATTCAGAATTANNNACATCTTTCTCGAAAACCTTCATTGC

CTATTTGATGTGTTTCCCCAAATCTCTGAATGGGAAATGTTGACAGGGATTTT

CCATGGTTATTTCTCCAATTTTCTCCAATTTTCTCCTTCTACAAAGTAACACAC

ACACACACACACACACACACACACACACACGCACACACACAGAGGAACTCC**GG**

AAAAGTCATTGGTT

(III) SAM25 (SAMPLE 25)

TTTGTCTTTCTCTGTCTCACTTATTTCACTTGTATGACAATCTCTAGGTCCATC

CATATTACTGCAAATGGCATTACTTCATTCTTTTTTACAGCTGAGTAATATTCT

ANTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATACATGCACCACATCTT

CTTTACAGGATTTCCTTTTTTTTTTTTAACAGCTTCATTGAGGTATAACTGATA

CAGAGTAGAAGTTGTACATGTTTGATATACACAATTTGGTAAGTTTGGCATGT

GCATGTTATGAGCTTGACATGTGCATTATCAACCCATGATGGGTACCGAGCTC

GAATTCCGTAATCATGGTCATAGCTGTTTCCTGTGTTGAAATTGTTATCCGCT

CACAATTCCACACAACATACGAACCCGGAAAGCATAAAGTGTTAAAGCCTGG

GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCT

TTCCANTCGGGAAANCTGTCGTGCCANCTGCATTAATGAACCGCCAACCCCC

NGGAAAAGCGGTTGCNTATTGGCGCTCTTCCGCTTCCCGCTCACTGAATCCCT

GCCCCCGTCCTTTCGGCTGCGGNAACGGTATCACTCACTCCAANGNNGNNAT

ACGGTATCCACAAC

(IV) RW4-1 (CLONE 4-1, SAMPLE 17)

CATNTCNACTCTAGAGGATCNCCCTAGCGAAAAAATGAAACAATGCCATTTG

CAGCACCACAGATGAGTCTAGGAGTAATCACGCAACTTGTGGTAAGTGAGAA

AGCGAAAGACACATGTCCTATCATATCACTTATAGGTGTTACCTAAAATGTG

ATACCAATGAAGATATTTCCACAGACAAAGGCAATCACAGATTCATTCAACA

AACTTACGGTTCACCCAATGGAAAGGTGTGAGGATTGGATACATTACGACAT

TGGGGTTAACAGAGATGTGGGTACCGAGCTCGAATTCGTAATCATGGTCATA

GCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAG

CCGGAAGCCATAAAGTGTTAAAGCCTGGGGTTGCCTAATGAGTTGAGCTAAC

TCCACATTAATTGCGTTTGCGCTCACTGCCCCGCTTTCCAGTTCGGGAAACCT

GTCGTGCCAGCTGCATTAATGAATCCGGCCAACGCCCCGGGAAAAGCGGTTT

GCGTTATTGGGCCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCNCTCCGTCGT

TCCGCTGCNGCGAACGGTATCACTCACTCCAAGCGGTAATACGGTTATCCAC

AGAATCNGGGATAACCCNGAAAAACATTTNAACCAAAGCCCNCAAAAGCCA

GAACCTTA

(V) RW4-25 (CLONE 4-25, SAMPLE 05)

AAATTGCTGTCATCTGGTCTGTCTGCTTCCTCTGCCTGAGGCCGAACTCACAT

GGGCAAGAACTTTCCCTGCAGTCATAGGAGGCTTCCATCCCACAAGAAGGTC

TCCTTAACATGGAAAGGCTCCCCCTGTTTTCCATCTTAGGGTTGTGTGTGTGT

GTGTGTGTGTGTGTGTGTGTGTGTGTTACAGGGAAGGGGTTGGANAGGGTGA

GGAATATATGTTTTAAAATCAAAAGTCCAATGCTTGGCTCTAGAACTTACCTG

CTGTTTAAACTTTGGATTCCCAAACACTGTTTGAGAAAGTCACTTCATGTCAC

AAAATGGCC

(VI) RW5-7 (CLONE 5-7, SAMPLE 36)

GATCNCCCTAGCGAAAAAATGAAACAATGCCATTTGCAGCACCACAGATGAG

TCTAGGAGTAATCACGCAACTTGTGGTAAGTGAGAAAGCGAAAGACACATGT

CCTATCATATCACTTATAGGTGTTACCTAAAATGTGATACCAATGAAGATATT

TCCACAGACAAAGGCAATCACAGATTCATTCAACAAACTTACGGTTCACCCA

ATGGAAAGGTGTGAGGATTGGATACATTACGACATTGGGGTTAACAGAGATG

TGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAA

TTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCCATAAAGTG

TTAAAGCCTGGGGTTGCCTAATGAGTTGAGCTAACTCCACATTAATTGCGTTT

GCGCTCACTGCCCCGCTTTCCAGTTCGGGAAACCTGTCGTGCCAGCTGCATTA

ATGAATCCGGCCAACGCCCCGGGAAAAGCGGTTTGCGTTATTGGGCCTCTTC

CGCTTCCTCGCTCACTGACTCGCTGCNCTCCGTCGTTCCGCTGCNGCGAACGG

TATCACTCACTCCAAGCGGTAATACGGTTATCCACAGAATCNGGGATAACCC

NGAAAAACATTTNAACCAAAGCCCNCAAAAGCCAGAACCTTA

(VII) RW5-12 (CLONE 5-12, SAMPLE 05)

TTTCCAGAACTGAAAGTAACCTGGTGTTACTGCATCAGAGGTTTTAAGTGTGT

GTGTGTGTGTGTGTGTGTGTGTGTGTGGTGTGGGGGGAAATGAGGAGATGAT

GGTATGAGATTAGATTAGATCAGTAAATGGGTGTCAGATGATTCAGAACCTT

GTATGCCATGGTTAAGACTCTANATTTTAAAATAAGTACAATGGAAACCAAT

CAAAAATTTAAGAGTAGAGAATCATTATCTGATTTAATTTTTAAGAGGGGTAC

CGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTAT

CCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCT

GGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCC

GCTTTCCAGTCGGGAAACCTGTCNTGCCAGCTGCATTAATGAATCGGCCAAC

GCCCNGGGAAAAGCGGTTTGCNTNTTGGGCGCTCTTCCGCTTCCTCGCTCACT

GACTCGCTGCGCTCGGTCGTTCNGCTGCGGCGAACGGTATCANCTCACTCAA

AGGCNGTAATACGGTTATCCNCACAATCCNGGGANAACCCCGGAAAAACAT

GTNAACAAAAGGCCCCAAAGGCCAGAACGTAAAAAGCCCCTTGCTGGCGTTT

TCCC

(VIII) RW5-5 (CLONE 5-5, SAMPLE 35)

GGTGGGGACGGANGGCTGGCAGGGACGTGGGGGGAAGGCTCTGGGAGTTCG

GGGAGGCTCTTCCGAGGGAGAGGTGGAAGTCGTCCCATCACGGGACTTTTTT

GCGGAGTTTTTGTTTGACATCTGATCTGGCGCGGGTCTCAGGAAGAANGGGG

CAGGTGGAAGTGTCGGGAGGGCAACGTTGACCTGACCTGCTCTCTCTTAGTTT

CCATCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTTGTGTCC

GTCCCCGTGTCCAGTCCAGGCTTCTGGCAGCCGCAGATGCCTTCAGTGGGGGT

ACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTT

ATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGC

CTGGGGTTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTG

CCCGCTTTCCANTCGGGAAACCTGTCGTGCCANCTGCATTAATGAATCCGCCA

ACNCCCCGGGAAAAGCCGTTTGCGTNTTGGGCGCTCTTCCGCTTCCTCGCTCA

CTGACTCCCTGCGCTCGGTCNTTCGCTGCGCAACCGTATCACTCACTCAAAGC

GGTAATACNGTTATCCCNNAACANGGGATAACCCNGAAAAACCC

(IX) RW 4-24 (CLONE 4-24, SAMPLE 04)

TCTGATAGATCAAAGTACATGGGCCAGTGTGGAACCCANAACAGGATCAAGA

TGTAATGTACATCCANGGCGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT

GTGTATGTATCTTTTGTTTCAAAACTATTTTAAATCCAAATAATATCTCTATTG

CTTAGGAAAAATGTGATTAAAGAGTCCATTTTGAGACTATTGGTTAAGTGATA

AGTACATACCTATATTTATAATAATTTATATATACATATATATCATAAAATNN

NNATGATGAGAAATTTAGCATTTGTGTTTGAATACTTTATTTCTATATTTAAA

AGTTAAGATCTCCACAATTCAGGGCTTGGTTGGAGGAAACCCTTCAACAAAG

CCTGGCAGGGATTATTTCTTAGATTTTTAGAA

(X) RW4-11 (CLONE 4-11, SAMPLE 27)

GTCTTTCTCTGTCTCACTTATTTCACTTGTATGACAATCTCTAGGTCCATCCAT

ATTACTGCAAATGGCATTACTTCATTCTTTTTTACAGCTGAGTAATATTCTAGT

GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATACATGCACCACATCTTCTTT

ACAGGATTTCCTTTTTTTTTTTTAACAGCTTCATTGAGGTATAACTGATACAGA

GTAGAAGTTGTACATGTTTGATATACACAATTTGGTAAGTTTGGCATGTGCAT

GTTATGAGCTTGACATGTGCATTATCAACCCATGATGGGTACCGAGCTCGAAT

TCGTAATCATGGTCATAGCTGTTTCCTGTGTTGAAATTGTTATCCGCTCACAA

TTCCACACAACATACGAGCCGGAAGCATAAAGTTGTAAAGCCTGGGGTGCCT

AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAA

TCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCCGGGA

AAAGCGGTTTGCGTTATTGGGCGCTCTTCCCGCTTCCTCGCTCACTGACTCGC

TGCGCTCNGTCGTTCCGCTGCGGCGAACGGTATCAGCTCACTCCAAGGCGGT

AATACGGTTATCCACCC

(XI) RW13 (SAMPLE 01)

ANCCCAGCAGGTGAGCAGAAAGCAATTATTAACCCTTAGTGTAACGTTCTAN

AATTGTGCTGATAATTAATACATTTTCTCTATCTTTGAGAGTCAGAAGGATCC

CTGACATCAGGGCTCTACTCAGCAAATAGCCTAGAGAGGACCCAATGCCTCT

GAATTTATAGGAAAGTGTTTACAATAAAACTTGTGAAGTTTTGTTTTTGTTTC

ATCTTGGGAGAGACTATATGATACAAAGAAAGCTGGAGTGCTGTTTTAAACA

CAGGGGTGTGTGTTTGTGTGAGTTGTGAATGTGCCCANTGTGCGTTGTGGGTG

TGCTGTGAGCACATGGTTTCATTATGTGTGGGGTGTGTGTGTANTGTGTATGG

TGTGTGTGAGTGTGGTGTCTGAGTGTGTGGGGTTGATTGTATGGAAATTGTGG

GCATATGACTACATTGGGCATATAAGGGAATGTTTATGTGAGGTGTGTATGCC

NACTGTTGGGTGTGTGTGTGTGTGTATTTGTAAAATTACTGCTTTCTCCCACTT

TTACTGANGTGTAATTGACATACAGGGGTACCGAACTCGAATTCTAATCATG

GTCATAGCTGTTTCCTGTGTGAAATGGTTACCCGCTCACATTCCCCCAACATA

CACC

(XII) RW4-3 (CLONE 4-3, SAMPLE 19)

CCCCCCCCCTTCTGCATGCGGCTTGTCNACTCTACAGGATCCCCCNGTCAGAC

TCTCNACGTGGGCACTCGGATATTCCTTTAGATACACATCACCGGATCACAAT

AAAAGTTAGGAAATCTTCCAAACGATCGCAGGCAGACGCTCGGGGCACTTCA

ACTCTAAAGTGGGGCCCCCAAGGAAATCTTTCANAANAANTCCAGTTTGTGT

GTGTGTGTGTGTGTGTGTGAGANAGACAGTTTTTTCATTCTCTAAAAAATCTA

NTTTTTAAACAAAGATCAATGTGTTATGCTGAAGTGTTGAGTGACCAGACCTC

ACACTGGTCAGAATGGTCATCATTAAAAAGTCTATAAATAACAAATGCTGGA

GAGGGTGTGGAGAAAGGGGAACCCTCCTACACTATTGGTGGGAATGTAAGTT

GGTGCANCCACTATGGAAAACAGTATGGANGTTCCTCAAAAAACTAAAAATA

GAACTACCGTATGATCCAACAATCCCACTCCTGGGCATATATCTGGACAAAA

CCANAATTCAAAAAGATACATGTGGGTTACCGAGCTCGAATTCCGTAATCAT

GGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCCACCACA

TACGAACCCGAAGCCTAAAGTGTNAACCTGGGGTGCCTAATGAATGANCTAA

CTCACATTAATTGCGTTGCGCCCATGCCCCT

(XIII) RW4-19 (CLONE 4-19, SAMPLE 35)

TCTCTCTCTATGGACTGGGTGTGAAGGGAGTCACTACACACACACACAGACA

CACACACACACACCCCTCACCAGAAGATAATATGATAGGTGGCAAATAACAG

CACATGCTATTTTCCAAAGAAATCATCCTCACATAATCCTCTCTAGCCTCTGA

CAACTTGATCCTTTTTATTACCTTTGGTGGGAGATAGGGTCCAGTAGTTGAAT

ATTTCCTTTGAAAATCTGCATCTTGATATCTTAGAACTTATTTTGGTGTCTGAT

ATATCTTGGTGGGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTT

CCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAA

GCATAAAGTTGTAAAGCCTGGGGTTGCCTAATGAGTTGAGCTAACTCACATT

AATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGC

TGCATTAATGAATCGGCCAACGCCNCGGGGAAAAGCGGTTTGCGTTTTTGGG

CGCTCTTCCGCTTCCTCGCTCACTGACTCCCTGCGCTCCGTCGTTCNGCTGCN

GCGAACGGTATCAGCTCACTCAAAGCGGTAATACGGTTATCCACAGAATCAG

GGGATAACCCAGGAAAAACATGTNANCCCC

Appendix three

List of mother-calf pairs included in paternity analysis (N = 62). Paternity assignments are included from analysis using a 0% mismatch error rate for males over nine years and males of all age. Paternity assignments are presented for analysis based on a 3% mismatch error rate.

Calf	Mother	Top Male Ia[1]	Top Male Ib [2]	Non-excl.	Calf Nursery	Match Prob.[3]	All age males [4]	Top Male II	Match Prob.
1123	1142	1819	xxx2	11	bof	ns	nc		
1163	1135	xx11		2	bof	80	nc	1170	ns
1170	1171	1616		7	bof	ns	nc		
1243	1242	1021		1	bof	95	nc		
1267	1266	1156		1	bof	95	nc		80
1301	1001				bof		nc	2410	ns
1311	1310	1805	1176	3	bof	ns	nc		
1402	1157	1320		2	bof	80	nc		
1405	1171	1155	1616	2	bof	ns	nc		
1406	1135				bof		nc	1130	ns
1408	1118	1327		2	bof	ns	nc		
1411	1142				bof		nc	1166	ns
1427	1168				bof		nc	1818.	ns
1429	1201				non		nc	1613	ns
1507	1266	1333		1	bof	95	nc		
1601	1281	1170	1616	10	bof	ns	nc	1174	ns
1607	1254				bof		nc	2310	ns
1608	1163				non		nc	1155	ns
1610	1509				*		nc	1821	ns
1702	1118	1041		1	bof	95	nc		
1703	1157	1516		1	non	95	nc		
1706	1135	1152		1	bof	95	nc		
1709	1127	1155		5	non	80	nc	1131	ns
1803	1266	1156		1	bof	95	nc		
1901	1201				bof		nc	1147	ns
1903	1303				bof		nc	1131	95
1911	1001	1017		7	bof	ns	nc		
1931	1301				bof		nc	1166	ns
1941	1241	2410		3	bof	80	nc	1803	ns
1954	1254	2340		1	bof	95	nc	1207	ns

1971	1171				bof		nc	1048	ns
2010	1310	1176		4	bof	80	nc	1176	ns
2018	1118	1716		1	bof	95	nc	1310/1716	ns
2027	1127	1130		4	bof	80	nc		
2029	1629				bof		nc	1320	ns
2135	1135	1156		5	bof	80	nc	2410	ns
2142	1242				bof		nc	1625	ns
2143	1243	1616		1	bof	80	nc		
2145	1145	1150		2	bof	80	nc		
2158	1158	1126	1805 tmp39	6	bof	ns	nc	1170	ns
2163	1163				bof		nc	1048	80
2201	1001				bof		nc	1245	ns
2209	1509	1310		10	non	ns	nc		
2215	1315	1323		1	non	95	nc		
2223	1223				bof		nc	1170	ns
2230	1430	2310	xx11	2	non		nc	1239/2310	ns
2427	1127				non		nc	1032	ns
2541	1241	1150	1156	3	bof	ns	nc		
2557	1157	1238		1	bof	95	nc		
2601	1701	1323		1	bof	95	nc		
2602	1602	1327		1	non	95	nc		
2605	1705				bof		nc	1623	ns
2608	1408				bof		nc	Tmp40	ns
2614	1114	1271		3	bof	ns	nc	Tmp39	ns
2615	1815	1155		1	bof	95	nc	1616	ns
2642	1142				bof		nc	1170	ns
2681	1281	1174		6	bof	ns	nc		
2704	1704				bof		nc	1804/1226	ns
2707	1707				bof		nc	1616	ns
2709	1509	1920		5	bof	80	2027	2027/1920	ns
2710	1004	1033		1	bof	80	nc		

2750	1950	1048	1122	3	bof	nc	1131	ns
Tmp20	1315				non	nc	1331	ns

[1]Top ranked LOD male (males of nine years or older). [2] Ties among top ranking males. [3]Match probability-significance of paternity assignment (ns = non-significant). [3]Top ranking male among males of all ages (nc = no change in paternity assignment relative to males over nine).