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Genetic kinship analyses of bowhead whales (*Balaena mysticetus*) sampled in Foxe Basin and Cumberland Sound, Nunavut, Canada

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Knowledge of the genetic relatedness between individuals, or kinship, can be a useful tool for research in behaviour, evolution and conservation especially in systems with limited genetic differentiation. Bowhead whales (*Balaena mysticetus*) in the eastern Canadian Arctic and western Greenland belong to a single population that is highly sex- and age-structured over its range. Kinship analysis of these different groups may provide information relevant to management considerations when addressing impacts of industrial development and other human activities on these whales. We analyzed biopsy samples of bowheads taken from summer aggregations (2008-2012) of animals in Foxe Basin and Cumberland Sound, Nunavut for patterns of relatedness and individual relationship categories. Overall, neither group of bowheads were composed of related individuals, though the relatedness measure for Foxe Basin was larger than that of Cumberland Sound. In addition, most parent-offspring pairs identified by the analyses involved whales sampled in Foxe Basin. These results provide genetic evidence to support the observations that Foxe Basin is an important nursery area for bowheads. The results of this study also demonstrate the potential to construct pedigrees from biopsy sampled whales in this population. Such pedigrees would have value for understanding bowhead dispersal, mating systems, reproductive success and other life history patterns in this population.

RÉSUMÉ

Analyses des indices de filiation génétique des baleines boréales (*Balaena mysticetus*) échantillonnées dans le bassin Foxe et la baie Cumberland au Nunavut (Canada)

La connaissance du niveau de parenté génétique, ou les indices de filiation génétique, qui existent entre les individus peut être un outil utile à la recherche du comportement, l'évolution et la conservation, notamment dans les systèmes où la différenciation génétique est limitée. Les baleines boréales (*Balaena mysticetus*) dans l'est de l'Arctique canadien et à l'ouest du Groenland appartiennent à une seule population fortement structurée en fonction du sexe et de l'âge dans l'ensemble de son aire de répartition. Les analyses des indices de filiation génétique de ces différents groupes pourraient fournir des renseignements utiles sur le plan de la gestion pour traiter les impacts du développement industriel et d'autres activités anthropiques sur ces baleines. Nous avons analysé des biopsies prélevés sur des baleines boréales pendant les regroupements estivaux (2008-2012) des animaux dans le bassin Foxe et la baie Cumberland, au Nunavut, en vue de relever les degrés de parenté et les différentes catégories de relations. Dans l'ensemble, aucun des groupes de baleines boréales n'était composé d'animaux apparentés, même si la mesure du degré de parenté dans le bassin Foxe était plus grande que dans la baie Cumberland. En outre, la majorité des paires parent/enfant identifiées par les analyses concernaient des baleines présentes dans le bassin Foxe. Ces résultats apportent des preuves génétiques à l'appui des observations selon lesquelles le bassin Foxe est une aire de croissance importante pour les baleines boréales. Les résultats de cette étude montrent également le potentiel d'établir des pedigrees à partir des biopsies prélevés sur les baleines de cette population. Ces pedigrees seraient utiles pour comprendre les schémas de dispersion des baleines boréales, les systèmes d'accouplement, le succès de la reproduction et d'autres éléments du cycle biologique de cette population.

INTRODUCTION

Bowhead whales (*Balaena mysticetus*) in the Canadian Arctic are considered to belong to a single population, the Eastern Canada-West Greenland (EC-WG) bowhead population (COSEWIC 2009). This designation was a change from the previous stock hypothesis that divided bowheads into two management units (Hudson Bay-Foxe Basin and Baffin Bay-Davis Strait) based largely on geographic discontinuities (Moore and Reeves 1993, Rugh et al. 2003). However, studies during the last decade using satellite tracking data of whales tagged in both Canada and Greenland have shown that bowheads move rapidly and freely between the hypothesized two stock areas (Heide-Jørgensen et al. 2003, 2006; Ferguson et al. 2010). Based on this information, the International Whaling Committee (IWC) provisionally revised the stock hypothesis for bowheads to reflect a single population model in 2007 which was then also adopted by COSEWIC in 2009. As of 2012, the IWC designation remains a provisional one, with requests for additional detailed genetic analyses still pending (IWC/64/Report 1 Annex F: Report of the Sub-Committee on Bowhead, Right and Gray Whales 2012).

Recent work has been done for the EC-WG bowhead population using population-based genetic inference methods (Wiig et al. 2011; McLeod et al. 2012; Alter et al. 2012), however the primary goals of these studies were not to assess a one-stock vs. two-stock hypothesis. Generally the outcomes of these analyses do support the single population model, but also highlight the presence of contemporary (i.e. recent, from an evolutionary perspective) and high gene flow between Western and Eastern Arctic bowhead populations (Alter et al. 2012) and that it is unlikely that any population of bowhead whales will be in mutation-drift equilibrium (McLeod et al. 2012). These factors make it difficult to define genetic management units that are based on the amount of genetic divergence between populations (Palsbøll et al. 2006).

Kinship-based analyses of genetic data can be used to complement population-based methods for examining population genetic structure (Palsbøll et al. 2010). Two approaches are used to perform a kinship-based analysis: estimation of relatedness, which is a measure of the fraction of alleles shared identical by descent (IBD) among individuals; and the assignment of pairs or groups of individuals to categories of relationship (Blouin 2003). Parentage and kinship analysis are similar to mark-recapture studies and may be useful for examining gene flow and dispersal on a contemporary time scale (for example, among cohorts, years, or seasons) (Christie 2010).

In whales, analyses of relatedness and parentage have been most broadly used to study populations of delphinids, though even here, this is still a relatively new approach (Möller 2012). This perhaps makes sense as delphinids are generally the most social group of cetaceans. Kinship analyses involving the killer whale (*Orcinus orca*), for example, have informed studies on social structure, gene flow, dispersal, foraging behaviour, population structure, paternity, and male reproductive success (Pilot et al. 2010; Ford et al. 2011; Möller 2012). Social structure and association analyses using estimations of relatedness have been used most commonly for studies of humpback whales (Valsecchi et al. 2002; Pomilla and Rosenbaum 2006; Pierszalowski et al. 2013) and sperm whales (Richard et al. 1996; Mesnick 2011; Pinela et al. 2009; Ortega-Ortiz et al. 2012) and have addressed questions about kin selection, maternal care and migration patterns. A recent study of beluga whales (Colbeck et al. 2012) showed that belugas from three stocks of whales in eastern Canada migrate together with family groups but these networks largely dissociate in the summering areas (with the exception of females and offspring). This study helped to clarify how migration routes and site fidelity are being maintained for summer aggregations of these whales.

Observations and satellite tracking studies of bowhead whale movements in the EC-WG population also show predictable aggregations of animals (Heide-Jørgensen et al. 2003, 2006;

Ferguson et al. 2010). Furthermore, it has been shown that there is significant seasonal sex and age-class segregation of animals over their range (Reeves et al. 1983; Heide-Jørgensen et al. 2010). Whales summering in northern Foxe Basin appear to be mostly juveniles and adult females with calves, while other adult animals seem to be found in West Greenland in the spring and Isabella Bay in the fall (Finley 1990; Cosens and Innes 2000; Cosens and Blouw 2003; Heide-Jøregensen et al. 2010). Also, considering the variability in diet composition among groups of whales, Pomerleau et al. (2012) suggested that bowhead whales as a population may be considered generalists, but at the group level particular bowhead whales appear to specialize on a subgroup of zooplankton species. Currently, it is not known if any form of social structure exists within these groupings of animals.

The objective of this study was to test the hypothesis that whales aggregating in different areas in the summer (Foxe Basin in July and Cumberland Sound in August) exhibit differences in social structure and therefore would have differing patterns of relatedness. This preliminary kinship-based analysis is one part (along with genetic capture-mark-recapture (Petersen et al. 2014) of an investigation on the utility of genetic tagging to contribute to population assessments of the Eastern Canada-West Greenland bowhead whale population.

METHODS

GENETIC IDENTITY

Samples and data for genetic analyses

Bowhead skin samples were collected from free-ranging whales during satellite tagging studies and biopsy programs. Samples in the field were preserved either in a salt-saturated 20% DMSO solution (Seutin et al. 1991), flash frozen in liquid nitrogen, in RNAlater (Qiagen Inc., Valencia, CA, USA), or in Allprotect (Qiagen Inc., Valencia, CA) and frozen upon arrival at the lab. Field notes detailing the particulars of each animal sampled (e.g. relative size, associations such as group and cow-calf pairs) were recorded for most of the samples.

Total cellular DNA extractions were performed using a variety of methods including phenol:choloform (Amos and Holzel 1991), Qiagen spin columns (DNeasy Blood and Tissue kits), and the Biosprint automated platform (Qiagen Inc, Valencia, CA, USA). Molecular determination of sex was completed using methods described in Shaw et al. (2005) and Rosel et al. (2003). In many cases, these different methods were used in tandem to verify that correct sex information was resolved from the samples.

A total of n=404 biopsy samples from Foxe Basin (2009 – 2011) and Cumberland Sound (2001-2012) were used for this study. These samples were processed in the lab using a consistent set of laboratory methods and alleles called by a single individual with a subset of allele calls confirmed by a second individual.

Mitochondrial DNA sequencing

Mitochondrial DNA haplotypes were generated using 474bp of sequence in the mtDNA control region. Primers Belmt-5 (GAT AGA GTT TTT TGA GCC CG) and Belmt-6 (TCA CCA CCA ACA CCC AAA G) were used in a target polymerase chain reaction (PCR) mixture containing 1x iProof HF buffer containing 20mM $MgCl_2$ (BioRad Laboratories, Hercules, CA, USA); 10mM dNTP mix; 20µM of each primer; 0.5units of iProof High Fidelity Taq polymerase (BioRad Laboratories, Hercules, CA, USA); and approximately 50-500ng of template DNA. The PCR profile was as follows: 98°C for 3min.; 35 cycles of 98°C for 10s, 58°C for 45s, 72°C for 1min; extension at 72°C for 10min. Products were visualized using agarose gel electrophoresis and

successfully amplified samples were cleaned using RapidTip PCR clean-up columns (Diffinity Genomics, West Henrietta, NY, USA). DNA sequencing was performed using BigDye ver3.5 (Applied Biosystems) with the Belmt-6 primer as the sequencing primer (2uM). The PCR reaction was: 96°C for 1min.; 32 cycles of 96°C for 10s, 50°C for 30s, and 60°C for 4min; and an extension at 72°C for 7min. Sequencing was performed on an Applied Biosystems 3130xl genetic analyzer (Life Technologies).

DNA sequences were aligned and edited using MEGA ver.5 (Tamura et al. 2011) and haplotypes identified using GenAlEx ver.6.5 (Peakall and Smouse 2012).

Nuclear DNA microsatellites

Samples were genetically profiled using 17 microsatellite loci developed for bowheads (Huebinger et al. 2008) and 9 loci developed for other cetaceans (Valsecchi and Amos 1996; Buchanan et al. 1996; Waldick et al. 1999; and Palsbøll et al. 1997) (Appendix 1). Loci were amplified individually or in multiplexes in reactions with a total volume of 10uL. Reaction mixtures contained 1X GenAmp buffer II (Life Technologies Corporation, Carlsbad, CA, USA); 2.0mM $MgCl_2$; 0.2mM dNTPs; a variable amount of each primer (Appendix 1); 0.5units of AmpliTaq Gold Taq polymerase (Life Technologies Corporation, Carlsbad, CA, USA); and 10-150ng of template DNA. Thermal cycling profiles for all panels were 95°C for 1min.; 35 cycles of 95°C for 30s, annealing temperature for 30s, 72°C for 30s; extension at 72°C for 30min. Amplification products were analyzed using an Applied Biosystems 3130xl genetic analyzer (Life Technologies) with an internal Liz600 size standard (Life Technologies Corporation, Carlsbad, CA, USA. Alleles were scored according to size in base pairs using GeneMarker ver.2.4.3 software (SoftGenetics).

GENETIC DATA ANALYSES

Nuclear DNA

Error rate was determined through the use of replicate samples. Five positive control samples were profiled between three and eight times in independent reactions of the complete microsatellite set. A further 34 samples were profiled twice in independent reactions. The error rate was calculated as the number of allele changes per number of alleles typed for each locus. In addition, each plate of samples that was analyzed (containing approximately 96 samples) contained four to six positive controls from two individuals as well as negative controls for DNA extractions and PCR reaction mix. Error rates were quantified as the mean number of errors per locus and averaged over all loci used in the analyses. Genotyping errors due to typographic error, scoring of stutter peaks, nonamplified alleles (null alleles) and large allele dropout were assessed using MICRO-CHECKER ver.2.2.3 (Van Oosterhout et al. 2004).

The entire dataset was examined for matching samples using GenAlEx ver6.5 (Peakall and Smouse 2012). Loci were evaluated for deviations from Hardy-Weinberg expectations and for linkage disequilibrium using GENEPOP ver.4.0 (Rousset 2008) both globally and within the two locations. The following measures were calculated to evaluate the genetic variability within sample groups: observed and expected heterozygosity; number of alleles and allelic richness; and inbreeding coefficient. These measures were calculated in the GenAlEx ver6.5 (Peakall and Smouse 2012). Calculation of frequency based measure of differentiation among groups (F_{ST}) was conducted using Arlequin ver.3.5 (Excoffier and Lischer 2010).

Mean relatedness among individual samples within and between locations was estimated using the Queller and Goodnight (1989) regression-based estimator and the Lynch and Ritland (1999) correlation-based method-of-moments estimator as implemented in GenAlEx ver6.5 (Peakall

and Smouse 2012). Maximum likelihood estimates of relatedness and relationships were further tested using ML-RELATE (Kalinowski et al. 2006). For this analysis, loci identified as having null alleles were left in the analysis and alleles frequencies corrected for their presence (Wagner et al. 2006). Dropping the loci from the analysis can significantly alter the likelihoods of competing relationships and degrade the performance of the estimators (they are working with less information).

RESULTS

DATA QUALITY

The dataset was immediately reduced from 26 loci to 20 loci due to poor amplification (Bmy2, FCB11), too much missing information (RW31, GATA098), possible mutation (EV37) or other issues (EV1). Percent error was negligible in that no errors detected in samples replicated once and a very low rate of dropout (0.024) was observed in positive control samples that were replicated three or more times. Samples with genotypes missing >20% of allele information were then removed from the dataset. This left 387 samples in total for further analyses.

Estimating relatedness correctly requires that datasets satisfy certain requirements for data quality (Van de Casteele et al. 2001). Genotyping errors must be negligible, the presence of null alleles should be evaluated and corrected for if necessary during analyses (Wagner et al. 2006), loci used should not be under selection, loci with mutations should be removed and at the population level, testing for random mating, allele frequencies and linkage disequilibrium should be done (Van de Casteele et al. 2001). Results from data analyses with MICRO-CHECKER indicated that unusual alleles due to typos in data entry were not present and there was no evidence of large allele dropout. Systematic deviations from Hardy-Weinberg equilibrium were also not detected, but four loci showed evidence of null alleles. Further tests for deviations from Hardy-Weinberg equilibrium in GENEPOP ver.4.0 (Rousset 2008) revealed that these were not global departures.

Fairly large numbers of loci (30-40) are generally needed to have moderate confidence around a single pairwise estimate of relatedness (Blouin 2003), but fewer loci are often used especially if they have a relatively large number of alleles (Van de Casteele et al. 2001). The 20 loci remaining in our dataset satisfy this requirement in that all loci were highly polymorphic and individuals had high heterozygosity (Table 2).

Forty-six samples were removed from the dataset after sample pairs with matching genotypes were identified. These involved within-year recaptures and between-year recaptures at each location, but no recaptures were detected in this analysis between Foxe Basin and Cumberland Sound (note that these are 2008 – 2012 samples only). In the recaptures, individual animals were found to be sampled from two to four times. The final dataset for further analyses thus contained 343 samples genotyped at 20 loci. This sample set was comprised of 49% females and 49% males in Foxe Basin (2% not determined) and 43% females and 56% males in Cumberland Sound (1% not determined). These sex ratios are similar to analyses using samples from 1995 to 2007 and reported in Heide-Jørgensen et al. (2010).

PATTERNS OF RELATEDNESS

A population pairwise Fst comparison of the Foxe Basin and Cumberland Sound sample groups revealed no significant differentiation with an Fst of -0.00002 (*P* value = 0.4332 using 10000 permutations). An overall estimation of mean within group relatedness using the Queller and Goodnight (1989) estimator showed that samples from both Foxe Basin and Cumberland Sound did not differ from a random sample pulled from the population data (Figure 2). The same result

was found when analyzing females and males separately (not shown) and for samples compared by year and location (Figure 3). The Queller and Goodnight estimator is generally considered to provide the best overall estimate of relatedness (GenALEx manual), but has been shown to perform best when the population being tested contains at least 50% of related pairs (Van de Casteele et al. 2001). For tests of species groups with 60 or 70% of unrelated pairs and all mammal species tested, Lynch and Ritland's estimator was found to perform the best (Van de Casteele et al. 2001). However, the Lynch and Ritland estimator generally needs a large number of loci and has problems with highly polymorphic loci used with highly related groups (Blouin 2003). An analysis of the bowhead data with both estimators yielded the same results (data not shown).

Estimates of individual sample pair relationships were tested over 58,653 pair comparisons: 90.3% of pairs had the highest likelihood of an unrelated relationship; 9.5% with half-sibling relationships; 0.17% with full-sibling relationships; and 0.026% with parent-offspring relationships. This resulted in 15 pairs of samples in which a parent-offspring relationship was consistent with the genetic data at 95% confidence (Table 2). There were two pairs, however, where other relationship types were also supported with high confidence. Of these 15 parent-offspring pairs, 60% (9/15) involved both individuals having been sampled in Foxe Basin (FB). Excluding the pairs that field notes identified as cow/calf pairs (4 pairs), in 4/5 of the remaining FB-FB pairs, individuals were sampled between years and in 1/5 pairs individuals were sampled in the same year. Between location parent-offspring relationship pairs were identified in 33% (5/15) of pairs and within Cumberland Sound only one pair with a parent-offspring relationship was identified (1%). Also in the 15 parent-offspring pairs, 53% (8/15) were female-female pairs, 47% (7/15) were female-male pairs and no male-male parent-offspring pairs were found.

Combination of these parent-offspring relationship estimates with information on sex of the individuals, mitochondrial DNA haplotypes and field notes shows the promise and the limitations of this type of analysis (Table 3). Eleven of the 15 parent-offspring relationships identified in the analysis were supported by both the mtDNA haplotypes and the field observation notes. Two of the three samples that field notes identified as being from a cow/calf pair were found in the analysis (pairs #7 and #9). However, for cow/calf pair # 8, mtDNA haplotypes do not support the estimation of parent-offspring relationship for these individuals. It is possible that there is an error in haplotype identification and also that the field notes were recorded in error. The sampling of this pair did occur in a short time period on the same day when several cow/calf pairs were sampled. In this group of samples, replicates of both an adult and a calf were found during the genetic match analysis. It is possible that samples were confused during the recording of the field notes.

Sample IG09-57 (single, juvenile female, haplotype B002) was estimated to have a parent-offspring relationship with two different samples (pairs #1 and #2), one a male and one a female. Haplotypes and notes support the possibility that this is a pedigree group involving a mother, father and daughter all sampled in different years. However, manual comparison of the genotypes for these samples did not support this grouping. Instead, the genotypes supported a mother-daughter relationship between IG09-57 and IG11-022 and the alternate relationship of half-sibling between IG09-57 and IG12-027.

Sample IG12-095 (a large cow travelling with a calf, calf not sampled) was also involved in two parent-offspring pairs, both males sampled in Cumberland Sound in 2012 (pairs #10 and #11). Again, the haplotypes and field notes support the possibility that the mother of the calf was also the mother of the two males. Review of sibling pairs identified by the analyses (data not presented) did indicate these two male samples (PG12-010 and PG12-018) as half-siblings.

Not all of the possible parent-offspring pairs are supported by the haplotype and/or field note information. Pair #13 is a female pair with different haplotypes, which would nullify this result. However, there are two possible explanations for this. First, the haplotype identifications may contain an error and both samples should be reanalyzed for mtDNA sequence information to check. Second, the confidence analysis for this pair did indicate that a full-sib or half-sib relationship was also likely. Either of these could be valid given the haplotypes if the two females had a different father. Another female (PG12-113) was identified as part of two parent-offspring relationships (pairs #14 and #15) and these are both supported by the haplotypes. However, for pair #15, this relationship is not possible unless there has been an error in recording the field observations. It does not seem likely that a mother and calf would be sampled in different years in different locations.

DISCUSSION

While bowhead samples from both Foxe Basin and Cumberland Sound were found to be unrelated overall, the within group pairwise relatedness estimate was larger for Foxe Basin than Cumberland sound whales. In addition, a more detailed examination of pair relationships did reveal different patterns between samples from the two locations. The genetic confirmation of mother-calf associations and the higher percentage of parent-offspring relationships in the Foxe Basin samples support previous observations that this area is an important nursery area for bowheads (Cosens and Blouw 2003). However, there are not enough samples in this dataset to make any inferences about how long mother-offspring associations may persist in space and time.

Results from the pair relationship analysis, when combined with mtDNA haplotypes, field notes and manual comparisons of genotypes, indicate that some simple pedigree construction is possible with even a small sample of the overall bowhead population. Though no conclusions could be drawn from this analysis, the results are encouraging for the potential of this type of analysis and support the continuation of bowhead biopsy sampling.

The analysis in this study would benefit from the addition of other samples that would expand the temporal and spatial scope of pair comparisons. Microsatellite, mtDNA sequence and molecular sex data exist for samples collected from a broad area of the EC-WG bowhead population range (Wiig et al. 2011; McLeod et al. 2012; Alter et al. 2012). However, coordinating and collaborating for such an analysis would be time consuming and expensive. Other than additional samples, integration of satellite tracking and remote sensing data with kinship analyses could also provide insights on bowhead associations, particularly involving dispersal and foraging patterns (Ortega-Ortiz et al. 2012).

Additional samples will also necessitate the use of additional methods to assess kin-group structure. For example, statistical programs such as KINGROUP (Konolov et al. 2004) and COLONY (Wang and Santure 2009) implement maximum likelihood methods to detect both parental and sibling relationships and facilitate pedigree configurations.

Parentage and kinship analyses could provide useful information about mating strategies, reproductive success and dispersal patterns for EC-WG bowhead whales. However, this is largely dependent on being able to sample a larger proportion of the population (Palsböll et al. 2010). Especially for a long-lived species, an ongoing collection of population samples could potentially be used to reveal potential parents with putative offspring year after year (Pemberton 2008; Christie 2010). Such information could fill knowledge gaps in the understanding of life history and population assessments useful for informing management decisions.

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REFERENCES CITED

Alter, S.E., Rosenbaum, H.C., Rosenbaum, Postma, L.D., Whitridge, P., Gaines, C., Weber, D., Egan, M.G., Lindsay, M., Amato, G., Dueck, L., Brownell Jr., R.L., Heide-Jørgensen, M.P., Laidre, K.L., Caccone, G., and Hancock, B. L. 2012. Gene flow on ice: the role of sea ice and whaling in shaping Holarctic genetic diversity and population differentiation in bowhead whales (*Balaena mysticetus*). Ecol. Evol. 2: 2895-2911.

Amos, B., and Hoelzel, A.R. 1991. Long-term preservation of whale skin for DNA analysis. Rep. Int. Whaling Comm., Special Issue 13: 99-103.

Blouin, M.S. 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends Ecol. Evol. 18: 503-511.

Christie, M.R. 2010. Parentage in natural populations: novel methods to detect parent-offspring pairs in large data sets. Mol. Ecol. Res. 10: 115-128.

Colbeck, G.J., Duchesne, P.D., Postma, L.D., Lesage, V., Hammill, M.O., and Turgeon, J. 2012. Groups of related belugas (*Delphinapterus leucas*) travel together during their seasonal migrations in and around Hudson Bay. Proc. R. Soc. B. 280: 20122552.

Cosens, S.E., and Blouw, A. 2003. Size- and age-class segregation of bowhead whales summering in northern Foxe Basin: a photogrammetric analysis. Mar. Mamm. Sci. 19: 284-296.

Cosens, S.E., and Innes, S. 2000. Distribution and numbers of bowhead whales (*Balaena mysticetus*) in northwestern Hudson Bay in August 1995. Arctic 53: 36-41.

COSEWIC. 2009. COSEWIC assessment and update status report on the Bowhead Whale *Balaena mysticetus*, Bering-Chukchi-Beaufort population and Eastern Canada-West Greenland population, in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 49 pp.

Excoffier, L., and Lischer, H.E.L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10: 564-567.

Ferguson, S.H., Dueck, L.P., Loseto, L.L., and Luque, S.P. 2010. Bowhead whale *Balaena mysticetus* seasonal selection of sea ice. Mar. Ecol. Prog. Ser. 411: 285-297.

Finley, K.J. 1990. Isabella Bay, Baffin Island: an important historical and present-day concentration area for the endangered bowhead whale (*Balaena mysticetus*) of the eastern Canadian Arctic. Arctic 43: 137-152.

Ford, M.J., Hanson, M.B., Hempelmann, J.A., Ayres, K.L., Emmons, C.K., Schorr, G.S., Baird, R.W., Balcomb, K.C., Wasser, S.K., Parsons, K.M., and Balcomb-Bartok, K. 2011. Inferred paternity and male reproductive success in a killer whale (*Orcinus orca*) population. J. Hered. 102: 537-553.

Heide-Jørgensen, M.P., Laidre, K.L., Wiig, Ø., Jensen, M.V., Dueck, L., Maiers, L.D., Schmidt, H.C., and Hobbs, R.C. 2003. From Greenland to Canada in ten days:tracks of bowhead whales, *Balaena mysticetus*, across Baffin Bay. Arctic 56: 21-31.

Heide-Jørgensen, M.P., Laidre, K.L., Jensen, M.V., Dueck, L., and Postma, L.D. 2006. Dissolving stock discreteness with satellite tracking: bowhead whales in Baffin Bay. Mar. Mamm. Sci. 22: 34-45.

Heide-Jørgensen, M.P., Laidre, K.L., Wiig, Ø., Postma, L.D., Dueck, L., and Bachmann, L. 2010. Large-scale sexual segregation of bowhead whales. Endang. Species Res. 13: 73-78.

Jones, O.R., and Wang, J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol. Ecol. Res. 10: 551-555.

Kalinowski, S.T., Wagner, A.P., and Taper, M.L. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. Mol. Ecol. Notes 6: 576-579.

Konovalov, D., Manning, C., and Henshaw T. 2004. Kingroup: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. Mol. Ecol. 4: 779-782.

Lunch, M., and Ritland, K. 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152: 1753-1766.

McLeod, B., Frasier, R., Dyke, A., Savelle, J.M., and White, B.N. 2012. Examination of ten thousand years of mitochondrial DNA diversity and population demographics in bowhead whales (*Balaena mysticetus*) of the Central Canadian Arctic. Mar. Mam. Sci. 28: E426-E443.

Mesnick, S.L., Taylor, B.L., Archer, F.I., Martien, K.K., et al. 2011. Sperm whale population structure in the eastern and central North Pacific inferred by the use of single-nucleotide polymorphisms, microsatellites and mitochondrial DNA sequencing. Mol. Ecol. Res. 11 (Suppl. 1): 178-298.

Möller, L.M. 2012. Sociogenetic structure, kin associations and bonding in delphinids. Mol. Ecol. 21: 745-764.

Moore, s., and Reeves R.R. 1993. Distribution and movement. In: Burns, J.J., Montague, J.J. and Cowles, C.J. (eds) The bowhead whale. Soc. Mar.Mamm. Spec. Publ. 2: 313-378.

Ortega-Ortiz, J., Engelhaupt, D., Winsor, M., Mate, B.R., and Hoelzel, A.R. 2012. Kinship of long-term associates in the highly social sperm whale. Mol. Ecol. 21: 732-744.

Palsbøll, P.J., Bérubé, M., and Allendorf, F.W. 2006. Identification of management units using population genetic data. Trends Ecol. Evol. 23: 11-16.

Palsbøll, P.J., Peery, M.Z., and Bérubé, M. 2010. Detecting populations in the 'ambiguous' zone: kinship-based estimation of population structure at low genetic divergence. Mol. Ecol. Res. 10: 797-805.

Peakall, R., and Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research -an update. Bioinformatics 28: 2537-2539.

Pemberton, J.M. 2008. Wild pedigrees: the way forward. Proc. R. Soc. B. 275: 613-621.

Petersen, S.D., Johnson, L., LeBlanc, B., Higdon, J.W., Yurkowski, D., Matthews, C., Postma, L., and Ferguson, S.H. 2014. Preliminary investigation of genetic capture-mark-recapture to census bowhead whales (*Balaena mysticetus*) in Nunavut, Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/017. v + 9 p.

Pierszalowski, S.P., Ferrari, M., Glockner-Ferrari, D., Mizroch, S., Clapham, P.J., and Dickerson, B.R. 2013. Investigating the feasibility of using DNA from sloughed skin for individual identification and kinship analysis in humpback whales (*Megaptera novaiangliae*). Mar. Mam. Sci. 29: 533-541.

Pilot, M., Dahlheim, M.E., and Hoelzel, A.R. 2010. Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (*Orcinus orca*). J. Evol. Biol. 23: 20-31.

Pinela, A.M., Querouil, S., Magalhaes, S., Silva, M.A., Prieto, R., Matos, J.A., and Santos, R.S. 2009. Population genetics and social organization of the sperm whale (*Physeter macrocephalus*) in the Azores inferred by microsatellite analyses. Can. J. Zool. 87: 807-813.

Pomerleau, C., Lesage, V., Ferguson, S.H., Winkler, G., Petersen, S.D., and Higdon, J.W. 2012. Prey assemblage isotopic variability as a tool for assessing the diet and the spatial distribution of the bowhead whale *Balaena mysticetus* foraging in the Canadian eastern Arctic. MEPS 469: 161-174.

Pomilla, C., and Rosenbaum, H.C. 2006. Estimates of relatedness in groups of humpback whales (*Megaptera novaeangliae*) on two wintering grounds of the Southern Hemisphere. Mol.Ecol. 15: 2541-2555.

Queller, D.C. ,and Goodnight, K.F. 1989. Estimating relatedness from genetic markers. Evolution 43: 258-275.

Reeves, R., Mitchell, E., Mansfield, A., and McLaughlin, M. 1983. Distribution and migration of the bowhead whale, *Balaena mysticetus*, in the eastern North American Arctic. Arctic 36: 5-64.

Richard, K.R., Whitehead, H., and Wright, J.M. 1996. Polymorphic microsatellites from sperm whales and their use in the genetic identification of individuals from naturally sloughed pieces of skin. Mol. Ecol. 5: 313-315.

Rosel, P.E. 2003. PCR-based sex determination in Odontocete cetaceans. Conserv. Gen. 4: 647-649.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Res. 8: 103-106.

Rugh, D. DeMaster, D., Rooney, A. Briewick, J., Shelden, K., and Moore, S. 2003. A review of bowhead whale (*Balaena mysticetus*) stock identity. J. Cetacean Res. Manag. 5:267-279.

Seutin, G., White, B.N., and Boag, P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69: 82-90.

Shaw, C.N., Wilson, P.J., and White, B.N. 2003. A reliable molecular method for gender determination for marine mammals. J. Mamm. 84: 123-128.

Valsecchi, E., Hale, P., Corkeron, P., and Amos, W. 2002. Social structure in migrating humpback whales (*Megaptera novaeangliae*). Mol. Ecol. 11: 507-518.

Van de Casteele, T., Galbusera, P., and Matthysen, E. 2001. A comparison of microsatellite-based pairwise relatedness estimators. Mol. Ecol. 10: 1539-1549.

Van Oosterhout, C.V., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4: 535-538.

Wagner, A.P., Creel, S., and Kalinowski, S.T. 2006. Estimating relatedness and relationships using microsatellite loci with null alleles. Heredity 97: 336-345.

Wiig, Ø., Heide-Jørgensen, M.P., Lindqvist, C., Laidre, K.L., Postma, L.D., Dueck, L., Palsbøll, P.J., and Bachmann, L. 2011. Recaptures of genotyped bowhead whales *Balaena mysticetus* in eastern Canada and West Greenland. Endang. Species Res. 14: 235-242.

FIGURES AND TABLES

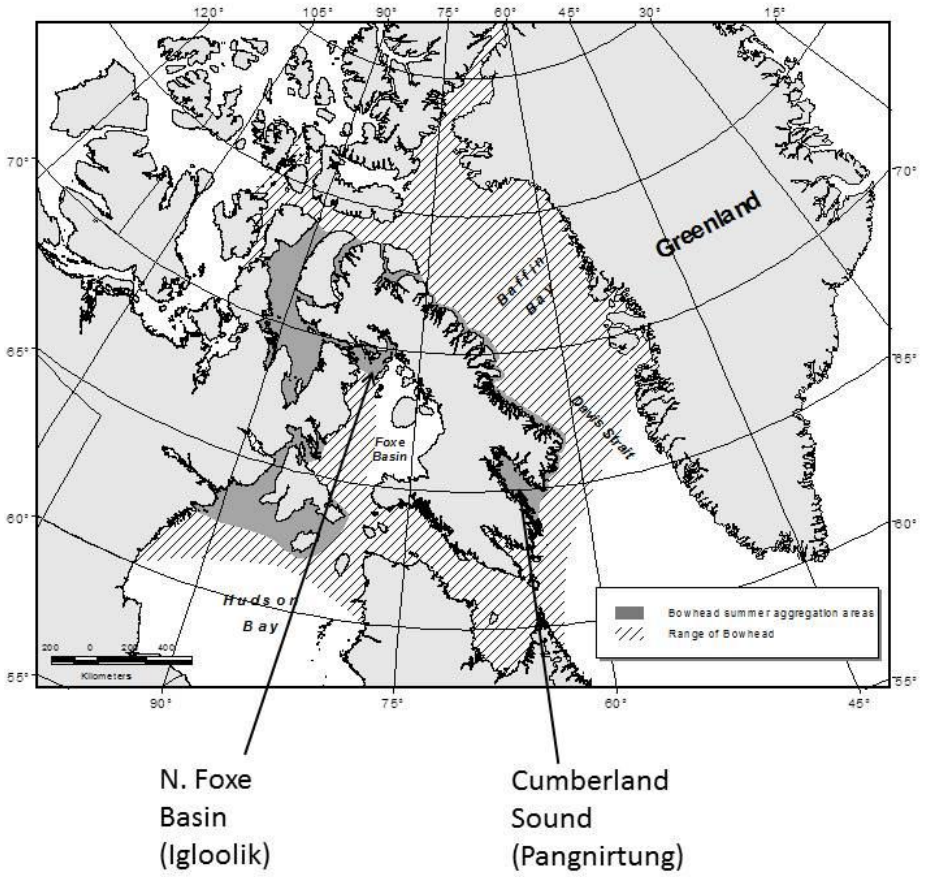

Figure 1. Locations of bowhead biopsy sampling in relation to the overall range and summer aggregations of bowheads in the EC-WG population. Sample ID numbers are assigned with a prefix designating the nearest community to where the sample was collected. These communities are indicated in brackets.

Table 1. Number of samples for each location analyzed in this study and sex ratio of samples.

Year	Foxe Basin (N)	F	M	Unknown	Cumberland Sound (N)	F	M	Unknown
2008	6	4	2					
2009	64	31	33					
2011	40	16	24		48	23	25	
2012	99	52	43	4	86	34	50	2
Total	209	103	102	4	134	57	75	2

Table 2. Allele information for marker loci used in this study. N = Sample size; Na = Number of alleles; Ne = Number of effective alleles; I = Shannon information index; Ho = Observed heterozygosity; He = Expected heterozygosity; uHe = Unbiased expected heterozygosity; F = Fixation index

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
Foxe Basin	**Bmy1**	201	10.000	5.139	1.810	0.806	0.805	0.807	-0.001
	Bmy10	207	21.000	12.992	2.722	0.937	0.923	0.925	-0.015
	Bmy11	205	13.000	7.563	2.126	0.849	0.868	0.870	0.022
	Bmy12	202	23.000	10.931	2.617	0.941	0.909	0.911	-0.035
	Bmy16	208	7.000	3.955	1.557	0.740	0.747	0.749	0.009
	Bmy19	177	13.000	6.302	2.105	0.859	0.841	0.844	-0.021
	Bmy26	207	21.000	13.036	2.723	0.942	0.923	0.926	-0.020
	Bmy33	207	10.000	3.875	1.627	0.763	0.742	0.744	-0.029
	Bmy36	198	25.000	16.087	2.901	0.965	0.938	0.940	-0.029
	Bmy49	203	22.000	9.086	2.565	0.877	0.890	0.892	0.015
	Bmy53	187	16.000	7.272	2.222	0.850	0.862	0.865	0.014
	Bmy54	209	7.000	3.980	1.582	0.746	0.749	0.751	0.003
	Bmy55	202	7.000	3.708	1.436	0.752	0.730	0.732	-0.030
	Bmy57	198	9.000	2.988	1.454	0.641	0.665	0.667	0.036
	Bmy58	202	24.000	13.482	2.831	0.926	0.926	0.928	0.000
	Bmy8	208	12.000	4.504	1.801	0.822	0.778	0.780	-0.057
	EV104	200	9.000	5.539	1.803	0.850	0.819	0.822	-0.037
	EV76	208	3.000	1.254	0.424	0.207	0.203	0.203	-0.020
	FCB4	198	16.000	3.991	1.846	0.747	0.749	0.751	0.003
	RW18	209	5.000	2.060	1.002	0.536	0.515	0.516	-0.042
Cumberland Sound	**Bmy1**	128	10.000	4.798	1.765	0.797	0.792	0.795	-0.007
	Bmy10	125	18.000	12.098	2.632	0.936	0.917	0.921	-0.020
	Bmy11	133	11.000	6.750	2.049	0.910	0.852	0.855	-0.068
	Bmy12	130	20.000	10.569	2.569	0.900	0.905	0.909	0.006
	Bmy16	134	7.000	4.365	1.640	0.784	0.771	0.774	-0.016
	Bmy19	114	13.000	7.063	2.176	0.921	0.858	0.862	-0.073
	Bmy26	130	18.000	12.408	2.654	0.938	0.919	0.923	-0.021
	Bmy33	96	9.000	4.044	1.639	0.656	0.753	0.757	0.128
	Bmy36	83	20.000	15.141	2.824	0.940	0.934	0.940	-0.006
	Bmy49	129	22.000	8.196	2.578	0.868	0.878	0.881	0.011
	Bmy53	100	15.000	7.840	2.272	0.790	0.872	0.877	0.095
	Bmy54	103	6.000	3.482	1.415	0.670	0.713	0.716	0.060
	Bmy55	130	7.000	3.598	1.426	0.654	0.722	0.725	0.095
	Bmy57	131	6.000	2.905	1.389	0.664	0.656	0.658	-0.013
	Bmy58	123	23.000	14.139	2.825	0.911	0.929	0.933	0.020
	Bmy8	134	13.000	4.578	1.855	0.754	0.782	0.784	0.036
	EV104	134	9.000	6.196	1.919	0.836	0.839	0.842	0.003
	EV76	131	3.000	1.316	0.486	0.260	0.240	0.241	-0.080
	FCB4	123	15.000	3.921	1.844	0.724	0.745	0.748	0.029
	RW18	134	5.000	2.390	1.122	0.582	0.582	0.584	-0.001

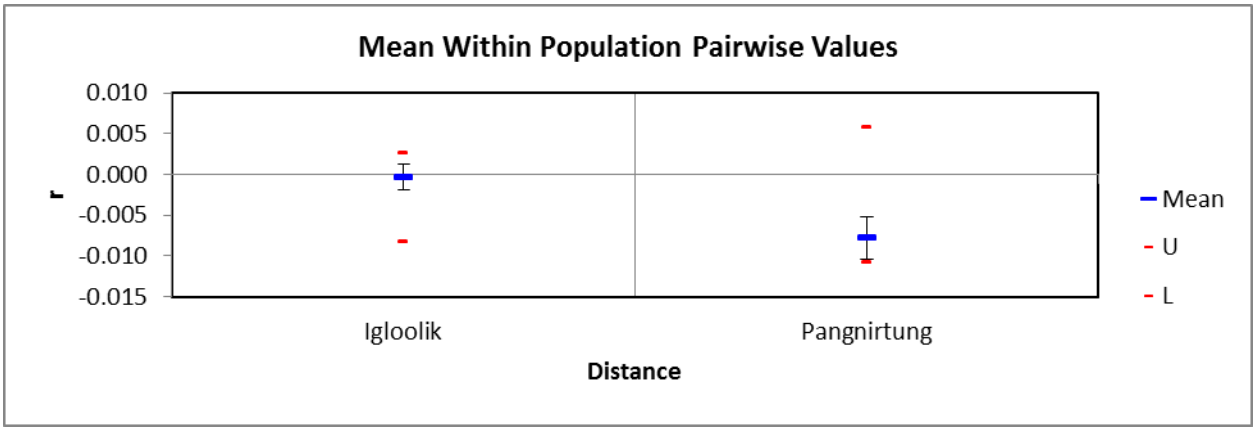

Figure 2. Mean within group relatedness of all samples grouped into sampling location. Confidence intervals (95%) obtained by permuting values around the null hypothesis of no differentiation from a relatedness of 0 (unrelated) are indicated with red bars. Confidence intervals (95%) around population means were obtained by bootstrap re-sampling.

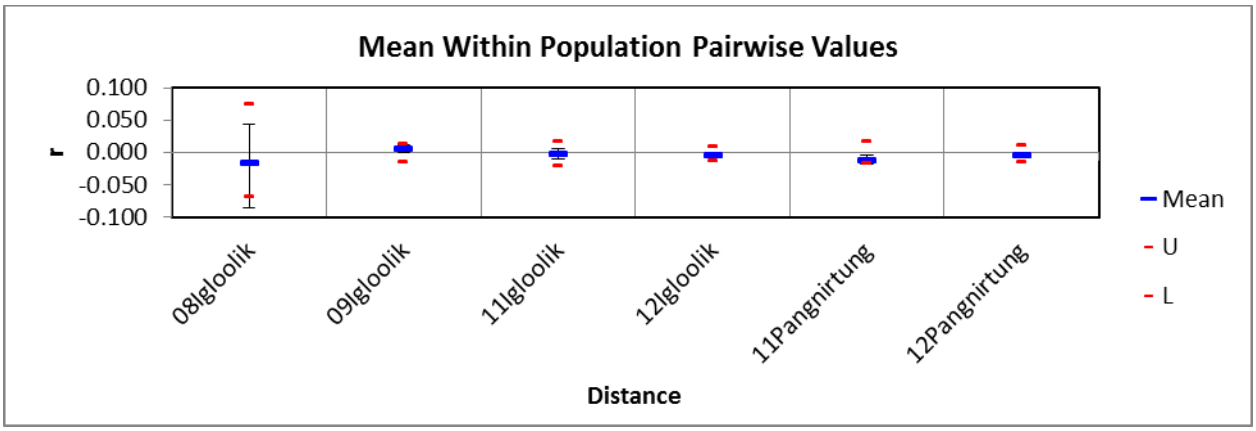

Figure 3. Mean within group relatedness of samples grouped by year and location. Confidence intervals (95%) obtained by permuting values around the null hypothesis of no differentiation from a relatedness of 0 (unrelated) are indicated with red bars. Confidence intervals (95%) around population means were obtained by bootstrap re-sampling.

Table 3. Pairs of individuals identified as having a Parent-Offspring (PO) relationship with the highest likelihood as estimated in the program ML-RELATE (Kalinowsky et al. 2006). Ind1 and Ind2 are sample IDs for the pair, R indicates the relationship with the highest likelihood, LnL(R) is the log likelihood of R, and R indicates which relationships are consistent with the genetic data with 95% confidence. Delta Ln(L) lists how much lower the log likelihoods are for the other possible relationships: U is Unrelated, HS is Half Sib, FS is Full Sib, PO is Parent Offspring.*

Ind1	Ind2	R	LnL(R)	R*	Delta Ln(L)			
					U	HS	FS	PO
IG11-022	IG09-057	PO	-91.6	PO	7.65	2.78	4.86	-
IG12-027	IG09-057	PO	-87	PO, HS	5.25	1.53	3.73	-
IG12-044	IG11-008	PO	-117.89	PO	20.94	6.77	4.02	-
IG12-045	IG09-082	PO	-100.88	PO	11.79	4.03	5.56	-
IG12-045	IG12-035	PO	-107.9	PO	12.88	4.43	6.1	-
IG12-050	IG09-073	PO	-99.12	PO	10.57	3.73	5.6	-
IG12-072	IG12-071	PO	-116.18	PO	12.98	4.15	5.55	-
IG12-090	IG12-089	PO	-118.24	PO	20.41	6.31	6.56	-
IG12-113	IG12-103	PO	-114.72	PO	18.91	6.43	3.65	-
PG12-010	IG12-095	PO	-98.71	PO	11.81	3.98	5.99	-
PG12-018	IG12-095	PO	-102.92	PO	14.01	4.46	5.29	-
PG12-032	IG09-055	PO	-86.24	PO	11.32	4.19	5.27	-
PG12-039	IG09-073	PO	-70.88	PO, FS, HS	6.74	2.11	1.15	-
PG12-113	IG11-004	PO	-77.24	PO	12.95	3.98	3.56	-
PG12-113	PG11-048	PO	-73.93	PO	13.36	4.03	5.72	-

Table 4. Estimated parent-offspring relationship pairs combined with the sex and haplotype of the sample and observation information about the sampled bowhead from field notes.

Pair #	Ind1	sex	haplotype	field notes	Ind2	sex	haplotype	field notes	R
1	IG11-022	F	B002	single, 9m	IG09-057	F	B002	single, juvenile	**PO**
2	IG12-027	M	B008	single, 11-12m, near socializing group (including 12m male tag#14497	IG09-057	F	B002	single, juvenile	**PO**
3	IG12-044	F	B019	10-12m, part of large group	IG11-008	F	B019	15m adult, tag#57600 accompanied by calf	**PO**
4	IG12-045	M	B002	10-12m, part of same large group as IG12-044	IG09-082	F	B008	11m, subadult	**PO**
5	IG12-045	M	B002	10-12m, part of same large group as IG12-044	IG12-035	F	B008	in group of 2, 13-14m	**PO**
6	IG12-050	M	B008	single, 9m	IG09-073	F	B008	in group of 4, large adult or subadult	**PO**
7	IG12-072	F	B008	calf, 6m, travelling with IG12-071	IG12-071	F	B008	adult, 14-15m, travelling with IG12-072	**PO**
8	IG12-090	F	B019	calf, 6m travelling with adult IG12-091(B019)	IG12-089	F	B002	cow, 13-14m, travelling with calf IG12-088 (B002)	**PO**
9	IG12-113	F	B002	calf, 5-6m, travelling with cow	IG12-103	F	B002	adult, 14m, travelling with calf	**PO**
10	PG12-010	M	B002	single, 10-11m	IG12-095	F	B002	Adult (cow), 13-14m, travelling with calf	**PO**
11	PG12-018	M	nd	in group of 3, medium size	IG12-095	F	B002	Adult (cow), 13-14m, travelling with calf	**PO**
12	PG12-032	F	B002	single, 10-11m	IG09-055	F	B002	adult (cow), travelling with calf	**PO**
13	PG12-039	F	B027	single, 13-14m, tag#114506	IG09-073	F	B008	large adult or subadult, in group of 4	**PO**
14	PG12-113	F	B034	no info	IG11-004	M	B034	in group of 2, 14m calf, probably with adult	**PO**
15	PG12-113	F	B034	no info	PG11-048	F	B034	PG11-047 (B002)	**PO**

APPENDIX

Appendix table 1. Details regarding PCR multiplexing and loci pooling strategies for bowhead. Refer to text for general reaction recipes and thermocycling conditions.

Panel	Locus	Label	Primer Concentration (μM)	Annealing Temperature (◦C)	Range	Primer Source
1	Bmy1	fam	1.6	50	243-263	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy8	vic	0.2	55	150-188	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy16	pet	0.6	50	210-222	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	EV37	fam	0.16	48/53	187-195	Valsecchi & Amos, 1996. Mol. Ecol. 5:151-156
	EV104	fam	0.04	48/53	147-165	Valsecchi & Amos, 1996. Mol. Ecol. 5:151-156
2	Bmy10	vic	0.4	50	212-256	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy55	fam	0.6	60	201-223	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	EV76	pet	0.2	48/53	152-162	Valsecchi and Amos, 1996. Mol. Ecol. 5:151-156
	FCB4	vic	0.3	48/53	150-206	Buchanan et al., 1996. Mol. Ecol. 5:571-575
	RW31	ned	1.2	55	122-132	Waldick et al., 1999. Mol.Ecol. 8:1753-1768
3	Bmy19	vic	0.15	60	104-132	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy33	vic	0.2	56	134-158	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy36	fam	0.3	56	141-197	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy53	vic	0.8	63	186-226	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy54	ned	0.2	63	157-169	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
4	Bmy49	pet	0.4	60	182-226	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy58	fam	0.4	60	127-187	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	RW18	vic	0.15	48/53	187-195	Waldick et al., 1999. Mol.Ecol. 8:1753-1768
5	Bmy11	fam	0.15	50	218-244	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy57	vic	0.06	60	152-166	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	EV1	fam	0.8	48/53	137-195	Valsecchi & Amos, 1996. Mol. Ecol. 5:151-156
single	Bmy2	vic	0.25	53	180-194	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy12	fam	0.25	53	120-166	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	Bmy26	ned	0.25	56	142-184	Huebinger et al., 2008. Mol.Ecol.Res. 8:612-615
	FCB11	pet	0.25	48/53	120-130	Buchanan et al., 1996. Mol. Ecol. 5:571-575
	GATA098	ned	0.25	48/53	86-110	Palsboll et al., 1997. Mol. Ecol. 6:893-895