POPULATION ESTIMATES OF MARK AND RECAPTURED GENOTYPED BOWHEAD WHALES (*BALAENA MYSTICETUS*) IN DISKO BAY, WEST GREENLAND

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ABSTRACT
Genotype and sex was determined for 342 individual (74 males and 268 females) bowhead whales (*Balaena mysticetus*) collected between 2000 and 2010 at Disko Bay in West Greenland. There were 21 between-year recaptures (four males and 17 females). A mark-recapture estimate of whales captured in 2010 and compared to all individuals captured between 2000 and 2009 resulted in an estimate of 1747 bowhead whales (SE=399, 95% CI: 966-2528) constituting the abundance of the spring aggregation in Disko Bay.

INTRODUCTION
In 1977, a two-stock hypothesis for bowhead whales occupying East Canada and West Greenland waters was adopted as the working model by the International Whaling Commission (IWC 1978). The stocks were: the Baffin Bay-Davis Strait (or Baffin Bay) stock and the Hudson Bay-Foxe Basin (or Hudson Bay) stock. The division was based on the disjunct summer distribution of bowhead whales in this region (see Reeves *et al.* 1983, Reeves and Mitchell 1990, Moore and Reeves 1993, Rugh *et al.* 2003). Bowhead whales are found in large numbers during the summer months at specific localities within Foxe Basin, Northern Hudson Bay, and in fjords along the east coast of Baffin Island and in the Canadian high Arctic. During the winter bowhead whale congregate in the Hudson Strait, at the mouth of Cumberland Sound, or along the West Greenland coast and in the North Water (Reeves *et al.* 1983, Reeves and Mitchell 1990). The International Whaling Commission has since 2007 revised the original two-stock hypothesis of bowhead whales in this region to a single stock hypothesis as the main working hypothesis, while acknowledging remaining uncertainties regarding the stock structure of bowhead whales in the area (e.g., IWC 2010).

Based on satellite telemetry data Heide-Jørgensen *et al.* (2006) argued that there are no obvious reasons for bowhead whales to restrict their movement to only parts of the total potential range in East Canada and West Greenland waters. Further, Heide-Jørgensen *et al.* (2010) and Wiig et al. (Submitted) argued that based on patterns of sexual aggregations and three occasions of recaptures between the two assumed stock areas of genotyped whales , bowhead whales summering in East Canada and wintering off the west coast of Greenland must belong to the same stock. In agreement with this, recent papers on satellite telemetry (Ferguson *et al.* 2010) and population genetics (Givens *et al.* 2010) have treated the bowhead whales in East Canada and West Greenland as one single stock.

Based on an aerial survey conducted in April 2006 Heide-Jørgensen et al. (2007) estimated that 1229 whales (95% CI: 495-2939) were present in Disko Bay at that time. The abundance was also shown to be increasing in West Greenland. In this paper we present and discuss genetic based mark-recapture data of bowhead whales sampled in Disko Bay between 2000 and 2010.

METHODS
Skin biopsies were collected from free-ranging bowhead whales in Disko Bay, West Greenland, between 2000 and 2010 using crossbows with biopsy darts (Palsbøll *et al.* 1991). The majority of samples were collected during field operations where bowhead whales were instrumented with satellite transmitters. Biopsies were also collected by local hunters who were asked to sample the whales. The majority of samples were collected between April and May. All samples were stored in saturated sodium chloride and 20% DMSO (Amos *et al.* 1992) and kept frozen at -20° C until genetic analysis.

Total genomic DNA was extracted from the skin biopsies using commercially available DNA extraction kits (DNeasy™ Blood and Tissue Kit [Qiagen], E.Z.N.A™. Tissue DNA kit [Omega Bio-tek], or GenElute™ [Sigma-Aldrich]) following the manufacturer's instructions. Molecular sex determination of all samples was conducted using a PCR-based approach following the principle outlined by Palsbøll *et al.* (1992) and the primers published by Berubé and Palsbøll (1996). In short, ZFX/ZFY PCR products were generated

using the primers *ZFYX582F* 5'-ATAGGTCTGCAGACTCTTCTA-3' and *ZFYX1204R* 5'-CATTATGTGCTGGTTCTTTTCTG-3' (Berubé and Palsbøll 1996). Subsequently, the PCR products were digested using the restriction endonuclease *Oli*I (Fermentas Inc.) that cuts at nucleotide position 152 in the SFX nucleotide sequences, thereby providing two fragments for females, only. The sex specific restriction fragment patterns were visualized by standard electrophoresis using 1% agarose gels and 0.5 x TBE buffer.

A 453 bp stretch of the mitochondrial (mt) control region, starting at position 15 473 and ending at position 15 925 in the GenBank Accession no. AP006472 (Arnason *et al.* 1993) was amplified as described by Borge *et al.* (2007). The obtained PCR products were purified using 10x diluted ExoSAP-IT (USB Corporation Inc.) following the manufacturer's instructions. Cycle-sequencing of the PCR products was conducted using BigDye™ 1.1 sequencing kit (Applied Biosystems Inc.) and the order of sequencing fragments resolved by using an ABI3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA). The collected DNA sequences were subsequently edited by hand with the software Sequencher 4.1 (GeneCodes) and manually with BioEdit (Hall 1999). The alignment of sequences was straightforward. The only ambiguity was one single-nucleotide insertion/deletion event in a homopolymer run of either five or six A's. All samples with identical mt control region sequence and sex were subsequently genotyped at four to eight highly variable microsatellite loci (Bmy26, Bmy29, Bmy33, Bmy38, Bmy41, Bmy42, Bmy51, and Bmy58) as described in Huebinger *et al.* 2006 to discern between different individuals. PCR products were separated and the length estimated using a ABI3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA) automated sequencer and scored using the software GeneMapper v4.0 (Applied Biosystems Inc., Foster City, CA, USA). Samples were inferred as originating from the same individual bowhead whale if they had identical sex, mitochondrial haplotype, and genotypes at minimum four microsatellite loci (for further details see Bachmann *et al.* in preparation).
The quality of the obtained molecular data was assessed by several control experiments. The molecular sexing was performed in duplicate for ~240 samples in the laboratories of the Natural History Museum, University of Oslo, Norway, and the Fisheries and Oceans Canada, Winnipeg, Manitoba, Canada, respectively, and no incongruence was detected. Accordingly, the error rate for the molecular sexing is less than ~0.5%. The mitochondrial control region sequences were determined for two independent DNA extractions of 48 samples, and no sequence differences were detected resulting in an error rate for the mt DNA sequencing at ~ 2% or less. For seven microsatellite loci (Bmy26, Bmy29, Bmy33, Bmy41, Bmy42, Bmy53, and Bmy58) a total of 460 gentotypes were determined on independent extractions, which yielded five different genotypes and an error rate slightly above 1%, assuming that at least one of any two disaccording genotypes was correct. Probabilities of two bowhead whale individuals sharing the same genotype (PI) for the scored microsatellite loci were calculated according to Paetkau & Strobeck (1994), and ranged between 7.09×10^{-3} (*Bmy29*) and 9.07×10^{-2} (*Bmy33*). The most conservative overall PI estimate (minimum criterion of four matching microsatellite genotypes only given the same sex and mitochondrial haplotype) was 6.85×10^{-6}.

A Chapman estimator with associate 95% CI (Chao and Huggins 2005) was used to make a mark-recapture estimate of the aggregation of whales in Disko Bay in 2010 assuming a closed population model. The population size was estimated as:

$$\hat{N} = ((n_1+1)(n_2+1)/(m_2+1)) - 1$$

and its variance as:

$$Var(\hat{N}) = ((n_1+1)(n_2+1)(n_1-m_2)(n_2-m_2))/(m_2+1)^2(m_2+2)$$

where $n_1$is the number of individuals captured 2000-2009, n_2 is the number of individuals captured in 2010, and m_2 the number of marked individuals captured in 2010. The 95% confidence interval (CI) was calculated as $\hat{N} \pm 1.96(Var\hat{N})^{-2}$.

Given the lifespan of bowhead whales, we assumed a zero mortality rate for all sampled individuals during the entire sampling period (2000-2010) so that all individuals identified in the period 2000-2009 were regarded as marked in the stock in 2010.

RESULTS

Genotype and sex were determined for 342 individuals (74 males and 268 females) (Table 1) sampled between 2000 and 2010. There were 21 between-year recaptures (four males and 17 females).

A mark-recapture estimate of the abundance in Disko Bay was calculated for individual whales identified and released between 2000 and 2009 (342) and re-identified in the sample of 75 with 13 recaptures collected in 2010. The resultant abundance estimate was 1747female bowhead whales (SE=399, 95% CI: 966-2528) in the Disko Bay aggregation.

DISCUSSION

The mark-recapture estimate of the bowhead abundance in Disko Bay in 2010 is apparently higher and more precise than the estimate of 1229 whales (95% CI: 495-2939) derived from an aerial survey conducted in April 2006 (Heide-Jørgensen et al. 2007). It is important to note that the aerial survey estimate covered a single snapshot in time whereas the mark-recapture estimate is based on 11 years of genetic identification of the whale abundance that supplies the aggregation in Disko Bay.

The Chapman mark-recapture estimator is based on a closed population model. The local aggregation of bowhead whales in Disko Bay constitutes just a fraction of the entire stock of bowhead whales inhabiting Eastern Canada and West Greenland, however, the mark-recapture method with genetic markers still provides a valid estimate of the local spring abundance of bowhead whales in Disko Bay. One assumption under this model is that all whales supplying the aggregation must have an equal probability of being sampled in at least the period with the initial identifications or in the sampling for re-identifications. The long-term (10 year) sampling for initial identification covers the purported bowhead reproductive cycle with cyclical returns to the Disko Bay (Wiig *et al*. submitted).

Another assumption of the closed model is that the stock size is constant over the study period. In our case the stock was likely increasing as observed by Heide-Jørgensen *et al.* (2007). This would negatively affect the abundance estimate of the aggregation in Disko Bay which consists of mostly mature females (Heide-Jørgensen et al. 2010). Very few males are observed in Disko Bay so the male proportion of the total stock is distributed in other areas (Heide-Jørgensen et al. 2010). Given that individual whales do not visit Disko Bay annually and that they are part of a larger stock, the mark-recapture estimate given here should not be viewed as an abundance estimate for the total stock of bowhead whales in eastern Canada and Baffin Bay. Bowhead might live more than 200 years (George *et al.* 1999) and it is possible that the area in Disko Bay frequented by bowhead whales during the spring is revisited by individual whales over longer intervals than the ten year period covered by the sampling at this locality. Thus, it is necessary to collect marks from whales a decade or more when using mark-recapture techniques to estimate the abundance of whales that supply the Disko Bay area in spring.

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Table 1. Number of individual male and female bowhead whales sampled each year, number of recaptures between years, and number of new individuals identified in Disko Bay, West Greenland, 2000 - 2010.

Year	Identified males	Recaptured males	New individual males	Identified females	Recaptured females	New individual females	New individual males and females
2000	5		5	1		1	6
2001	5		5	7		7	12
2002	4		4	6		6	10
2003	0		0	10		10	10
2004	0		0	1		1	1
2005	6		6	17	1	16	22
2006	0		0	20		20	20
2007	17	1	16	79		79	95
2008	10		10	34	1	33	43
2009	16	2	14	29	3	26	40
2010	11	1	10	64	12	52	62
Sum	74	4	70	268	17	251	321