

UPDATE OF MITOCHONDRIAL DNA ANALYSES ON STOCK STRUCTURE IN THE B-C-B STOCK OF BOWHEAD WHALE

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ABSTRACT

Genetic analyses based on mtDNA control region sequences were conducted on samples of the B-C-B stock of bowhead whale collected from different villages engaged in aboriginal whaling. Laboratory work on mitochondrial DNA sequencing was carried out by US scientists and access to these data was possible under the Scientific Committee's data access protocol (Procedure A). The total number of sequences available was 399. Approximately 68% of those come from a single locality (Point Barrow). An additional 24 sequences from the Okhotsk Sea bowhead whale stock were used in the analysis for comparative purposes. Same as previously reported, the nucleotide diversity was smaller in whales from the Okhotsk Sea stock than in whales from the different localities of the B-C-B stock. The quantification of the temporal and geographical mtDNA differentiation was carried out using the *F_{st}*. In addition the genetic relationship was quantified using the chi-square statistics for heterogeneity of mtDNA haplotype frequencies. First temporal (seasonal) differentiation was examined in those localities with larger sample sizes (St. Lawrence Island and Point Barrow). Subsequently geographical mtDNA differentiation among four localities (Point Barrow, St. Lawrence Island, Chukotka and Okhotsk Sea), was examined. No significant mtDNA heterogeneity was found among B-C-B stock whales, apart from a marginal significant seasonal heterogeneity for *F_{st}* found in St. Lawrence Island. In contrast with a previous mtDNA result, no significant heterogeneity was found at Barrow when the samples were grouped into spring and fall. Significant genetic heterogeneity was found between B-C-B stock localities and whales from the Okhotsk Sea stock. In general these mtDNA results provide no strong evidence for genetic population structure within the B-C-B stock. Sample sizes for several localities and months, however, remain low and therefore negative results in some comparison could just reflect the low statistical power of the analysis. Furthermore samples from Chukotka and St. Lawrence Island obtained in summer months, which are essential to investigate some of the multiple stock hypotheses, are not available because they are difficult to collect since there is no whaling at this time of the year. At Barrow nuclear DNA markers have suggested the possibility of additional stock structure and mixing of stocks. It is possible therefore that the mtDNA analysis could not identify structure if two stocks mix to each other in similar proportion when they pass through that locality in spring and autumn.

INTRODUCTION

For management purposes the IWC has recognized five stocks of the bowhead whale (*Balaena mysticetus*) (Fig. 1). All these stocks, but the Bering-Chukchi-Beaufort (B-C-B) stock, are in the category of heavily depleted (in some cases perhaps extinguished e.g. the Spitsbergen stock). The B-C-B stock is the target of aboriginal whaling and the IWC manages this stock under the Aboriginal Whaling Management Procedure (AWMP). During the 2002 Scientific Committee Meeting an estimation of 9,860 animals was given for this stock (IWC, 2003). The estimation come from sighting data obtained during the spring migration at Point Barrow.

The B-C-B stock winters in central and western Bering Sea. From April to June whales migrate north and east until they pass Point Barrow where they travel east toward the south-eastern Beaufort Sea. Whales spend most of the summer through the Beaufort Sea. During the fall whales migrate west out the Beaufort Sea. From mid-September to mid October bowhead whales are seen in the northeast

Chukchi Sea. Whales migrate from Point Barrow into the Chukchi Sea heading toward Wrangel Island. When they reach the Siberian coast, they follow it southeast to the Bering Sea.

Despite the limited availability of data and comparative analyses within the distribution area of the B-C-B stock, the Scientific Committee recommended in 2002 the single B-C-B stock scenario for management purpose (IWC, 2003). At that opportunity this was the only stock structure scenario considered by the AWMP. Recent genetic analyses conducted under the Scientific Committee data access protocol have showed substantial degree of heterogeneity within the B-C-B stock (e.g. Jorde *et al.*, 2004), and the possibility of additional stock structure has been considered. During the Second Intersessional AWMP Workshop for the 2007 Bowhead *Implementation Review*, a total of four stock structure hypotheses were adopted for use in the trials. Two of these hypotheses involve multiple-stock scenarios (IWC, 2007).

Previous mtDNA analysis showed a significant genetic heterogeneity when the samples in Point Barrow were grouped into spring (n= 98) and fall (n= 93) migrants ($F_{st}= 0.007$; $p=0.0403$) (Pastene *et al.*, 2004). Subsequent mtDNA analyses based on larger sample sizes (LeDuc *et al.*, 2006; Pastene *et al.*, 2007) failed to find any significant geographical or temporal genetic heterogeneity apart from a p-value smaller than 5% significant level when the samples from St. Lawrence Island were divided and compared for two temporal groups: April+May and Nov-Feb (Pastene *et al.*, 2007).

Interpretation of genetic results has been complicated because sample sizes are still in small numbers for some localities despite the considerable effort by US scientists to collect genetic samples for a wider range of the geographical and temporal distribution of the B-C-B bowhead whales. In addition it should be noted that the lack of samples from the breeding grounds preclude a comprehensive description of the stock structure for this species in this particular region.

The objective of this study is to conduct an update of the mitochondrial DNA (mtDNA) analysis similar to that conducted by Pastene *et al.* (2007) using the newest data set provided by US scientists (provided on 16 January 2007).

MATERIALS AND METHOD

Available data

Following the Procedure of Data Availability adopted by the Committee during the 2003 meeting (IWC, 2004), mtDNA data of the B-C-B stock was requested to and provided from US scientists. These included mtDNA control region sequences (397bp) of samples collected in different villages engaged in aboriginal whaling (Fig. 2).

Table 1 shows the number of samples available for the mtDNA analysis by locality and month. Most of the available samples from the B-C-B stock localities come from Barrow (approximately 68%). To make this data set comparable with that used by other analysts we made the same grouping of samples based on the supplemental data provided by LeDuc *et al.* (2007) (BRG 9) (see appendix). The following changes were made in relation to the data presented by these authors:

- i) In Barrow, we decided to delete data from a stranded animal (1BC97B) in August 1997 and a sample (05B12) from May 2005, which showed a 'Y' in the mtDNA sequence.
- ii) In Nuiqsut, we decided to delete data from a stranded animal (92N-BC-1) in September 1992.
- iii) In Pt. Hope two samples were deleted in May 2005 because of two duplicates (05H3_5 and 05H4_7) found in the latest data set.
- iv) In Gambell we deleted a sample in April 2003 (sample 03G1 was duplicated in BRG9). Also two samples were deleted in December 2002 because G-BAL-5 was not included in the latest data set and was represented twice in BRG9. We added two samples in 'UK' (G-BAL1_2 and GSK14) after examination of the latest data set.
- v) In Chukotka we added one sample in 'UK' (RUS-BW000824.19) after examination of the latest data set.

The hypothesis testing analysis followed a step wise fashion. First temporal (seasonal) variation was examined in St. Lawrence Island and North Slope (the localities with larger sample sizes). Analysis of yearly variation was not possible due to the small sample sizes obtained when the samples

were divided by year. However for the analysis of temporal (seasonal) variation the North Slope and Barrow samples were divided into two periods: 'Early Years' 1983-2000 and 'Late Years' 2001-2006. Next we conducted an examination of spatial differentiation among the main localities of the B-C-B stock: Barrow, St. Lawrence Island, Chukotka. For this analysis the total samples were used (included all samples in the 'unknown' category). Samples from the Okhotsk Sea were used as out-group.

mtDNA analysis

The evolutionary distance between two nucleotide sequences was calculated according to Kimura's two parameters method (Kimura, 1980). The degree of genetic diversity within each locality was estimated using the nucleotide diversity (Nei, 1987).

Following LeDuc *et al.* (2006) the F_{st} in AMOVA (Excoffier *et al.*, 1992) was used to investigate the temporal/spatial differentiation of mtDNA variation. In addition we used the randomized chi-square Test of Independence (Roff and Bentzen, 1989) to test genetic differences. In each test a total of 10,000 randomizations of the original data set were performed. A 'p' value below 5% was used as criteria for rejecting the null hypothesis of panmixia.

RESULTS

Variability of mtDNA control region sequences

A segment of 397bp of the mtDNA control region was determined in 399 animals. A total of 45 polymorphic sites defined 68 unique sequences (haplotypes) (Table 2). Nucleotide diversity by locality and stock is shown in Table 3. As reported by LeDuc *et al.* (2005) the diversity in the Okhotsk Sea samples was lower than in the B-C-B samples.

Geographical and temporal distribution of haplotypes

Table 2 shows the distribution of haplotypes among localities. Samples in Barrow were grouped into 'spring' and 'fall' groups. The samples from the Okhotsk Sea are included for comparison. The main haplotype in the B-C-B localities was haplotype '42' (26.3%) followed by haplotype '23' (7.3%). The main haplotype in the B-C-B localities was also the main haplotype in the Okhotsk Sea stock (54.2%). The four haplotypes in this stock were represented in the B-C-B localities.

Test for temporal differentiation

Table 4 shows the results of the heterogeneity test for temporal differentiation in St. Lawrence Island. The F_{st} showed marginal significant differences when the samples were grouped into spring and winter seasons.

Tables 5 and 6 show the results of the heterogeneity test for temporal differentiation in North Slope and Barrow, respectively. The seasonal differentiation analysis was conducted for 'Early Years' and 'Late Years' periods as well for the all years of sampling. None of the comparisons by chi-square and F_{st} resulted in significant mtDNA differences.

Test for spatial differentiation

Table 7 shows the results of the heterogeneity test for spatial differentiation. No significant differences were found among the three localities of the B-C-B stock. However significant differences were found when these localities were compared with whales of the Okhotsk Sea Stock. This differentiation was more evident from the results of the F_{st} analysis.

DISCUSSION

The analysis of mtDNA on B-C-B stock of bowhead whales conducted in this study failed to find evidence of significant genetic heterogeneity within this stock. The only evidence of heterogeneity was the results found for St. Lawrence Island where marginal significant mtDNA difference for F_{st} was found when the samples were grouped into spring and winter. It should be noted that in the previous analysis by Pastene *et al.* (2007) the p-value for this comparison was below 5% level. As suggested at that opportunity the sample sizes in this comparison is small, 11 and 13, respectively, and then no firm conclusion on stock structure can be obtained.

Our analysis was conducted for both sexes combined. An analysis by sexes in Barrow (locality with the larger sample size) had shown no differences between female and male samples (data not shown).

A strict analysis of yearly variation in the different localities was not possible due to the small sample size obtained when the samples were grouped by year. The analysis in Barrow was conducted for two year periods and no significant differences were found between these two periods. In both of these periods samples obtained in several years were combined. This required the assumption that the pattern of migration in each year is the same.

In general results of our mtDNA analysis provide no strong evidence for population structure within the B-C-B stock of bowhead whale. However the sample sizes for several localities and months remain low (see Table 1). Therefore the negative results of some of the tests could just reflect the low statistical power of the analysis. A sample size of n=24 in Chukotka was large enough to detect significant differences with the sample of the Okhotsk Sea stock. However the degree of genetic differentiation among putative stocks within the area of distribution of the B-C-B stock is expected to be smaller than that between B-C-B and Okhotsk Sea stocks, therefore larger sample sizes will be necessary to detect mtDNA differences among putative stocks within the B-C-B stock.

The Scientific Committee has recognized that the interpretation of the results of hypothesis tests when the null hypothesis is not rejected is difficult because failure to reject the null hypothesis may occur not only if the null hypothesis is true but also if the null hypothesis is false but the data are insufficient to identify this (i.e. the statistical test has low power). The Committee has recommended that the power of the statistical techniques should be explored in these cases. In the case of the North Pacific Bryde's whale RMP *Implementation* the genetic analysis involved the use of the same genetic markers but larger sample sizes by locality than in the case of the bowhead whale. No significant genetic differences among localities were found. Kitakado *et al.* (2005) evaluated power under an island model and the Workshop where the results were presented believed that the approach used in that study provided a defensible means of identifying the power of the hypothesis test (IWC, 2006). A similar approach could be used to examine the power of the hypothesis test in the case of the bowhead mtDNA analysis.

Apart from the issue of the number of samples, the attainment of samples in particular months is important to test different hypotheses on stock structure. For example samples from Chukotka and St. Lawrence Island obtained in summer months, which are essential to investigate some of the multiple stock hypotheses, are not available because they are difficult to collect since there is no whaling at this time of the year.

Previous mtDNA analysis showed a significant genetic heterogeneity when the samples in Barrow were grouped into spring and fall (Pastene *et al.*, 2004). However other mtDNA analyses based on larger sample sizes failed to find significant heterogeneity for this temporal comparison (LeDuc *et al.*, 2006; Pastene *et al.*, 2007). The present analysis corroborated the results of these authors. One explanation for these results is that there is no genetic structure of bowhead whale at Barrow. An alternative explanation for the negative results found is that whales from two different stocks are present in the spring and fall migrant groups but they mix in similar proportion in both seasons. The implication of this is that the occurrence of multiple stocks at that locality is not detected by the mtDNA analysis. A similar rationale has been presented in the case of the North Pacific Bryde's whale *Implementation* to explain negative results. In the case of bowhead whale, analyses of nuclear DNA markers in Barrow suggested the possibility of structure (see Kitakado *et al.* 2007) and the possibility of mixing can not be discarded. Apart from further analysis of nuclear markers during migration, the attainment of genetic samples from breeding grounds will help in resolving the issue of possible mixing of stocks during migration in the case of the bowhead whale.

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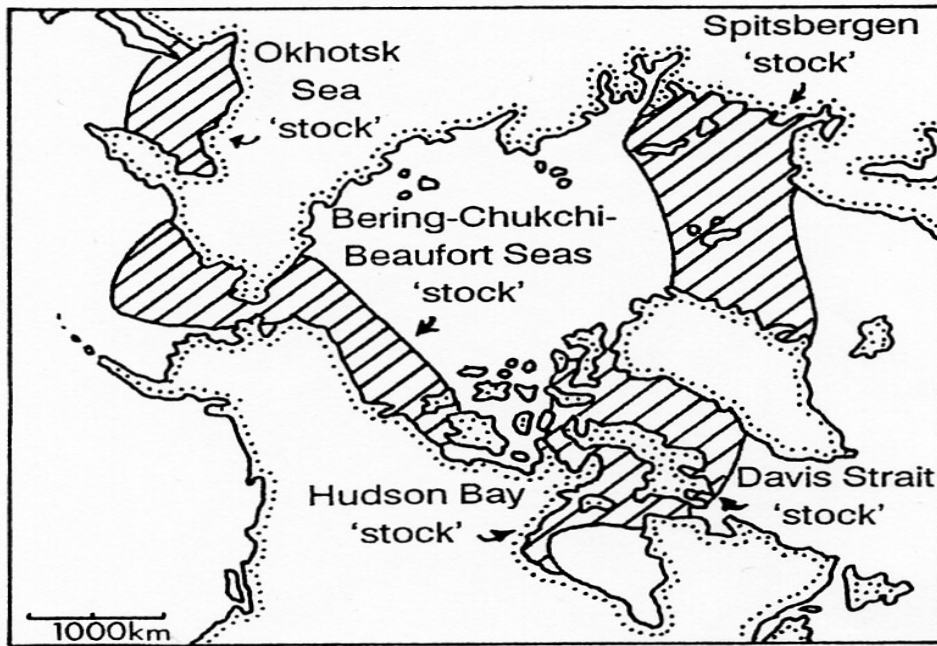


Fig. 1: Putative stocks of the bowhead whale (modified from IWC, 1992).

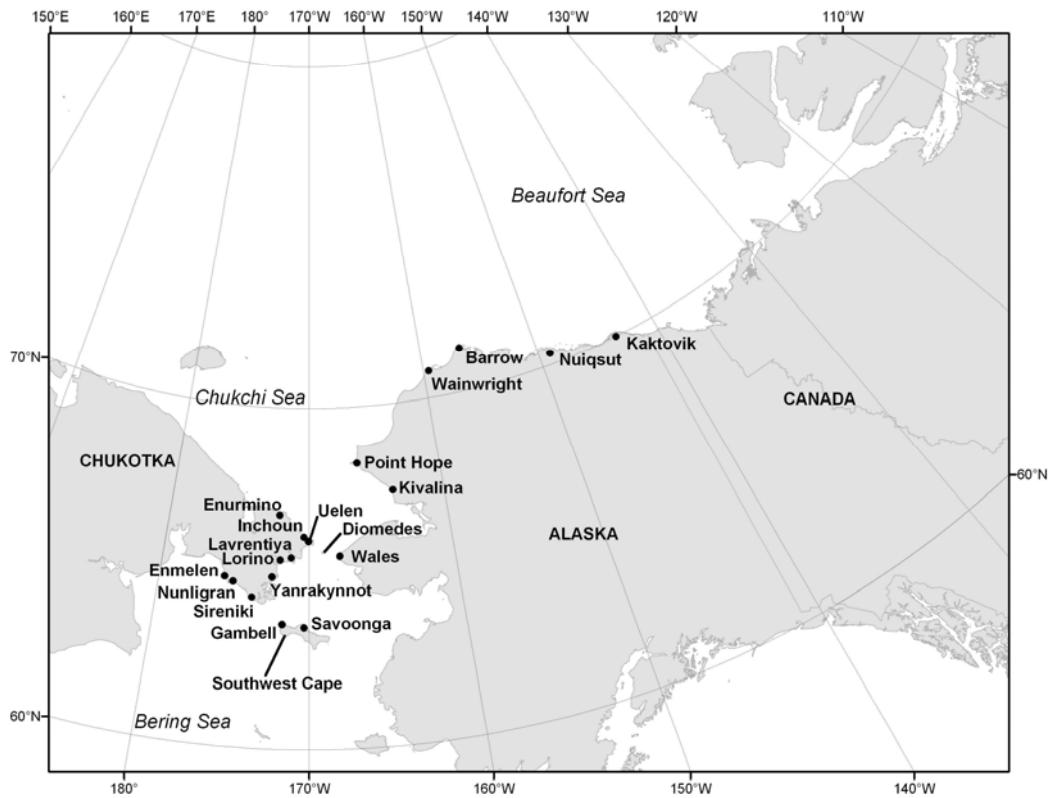


Fig. 2: Villages engaged aboriginal whaling of the B-C-B stock of bowhead whale. Genetic samples used in the present study were obtained from these villages.

Table 1: Number of samples of bowhead whale used in the present mtDNA analysis, by locality and month. All fetus samples except 99B6F are excluded from this Table. UK: sampled month or year is unknown. Grey color indicates differences in samples used regarding those in LeDuc *et al.* (2007).

Strata	Village	Year	Month												UK	Total		
			1	2	3	4	5	6	7	8	9	10	11	12				
	Barrow	1983					1											1
		1984					3											3
		1986				2	3											5
		1987					1											1
		1988										1						1
		1989					2						3					5
		1990					2						3					5
		1992					2				1	8	1					12
		1993				6	7											13
		1995					3	1				2	2					8
		1996				1	4					18						23
		1997					8	1				14	5					28
		1998					9					1	1					11
		1999				1	8						6					15
		2000				1	4					4	2					11
		2001				1	16						4					21
		2002					3					1	18					22
		2003				1	8	1					5					15
		2004				2	3	1				9	6					21
		2005				2	12					2	16					32
	2006					4											4	
	Total				17	103	4		1	60	72						257	
	Wainwright	1981					2										2	
		1984					1										1	
		2003				1											1	
		2005				2	2										4	
		2006					1										1	
	Total				3	6										9		
	Kaktovk	1986									2						2	
		1990									1						1	
		2000									3						3	
		2002									3						3	
		2003									2						2	
		2004									3						3	
		2005									3						3	
	Total									17						17		
	Nuiqsut	1992														0		
		2004									3					3		
		2005									1					1		
	Total									4					4			
	Pt.Hope	2003				3										3		
		2005				1	3									4		
		Total				4	3									7		
	Sub-total		0	0	0	24	112	4	0	1	81	72	0	0	0		294	

Table 1: continued

	1984			1										1
	1989				1									1
	1996			2										2
	2001								1					1
Savoonga	2002									3				3
	2003									2				2
	2005	1		3						3				7
	UK												3	3
	Total	1		6	1					4	5	3		20
	1996			1	1									2
	2002										1			1
	2003			1										1
Gambel	2004										1			1
	2005	1		1										2
	UK												30	30
	Total	1		3	1						2	30		37
Sub-total		2	0	0	9	2	0	0	0	0	4	7	33	57
	1998				2									2
	1999									1				1
	2000							1						1
	2001								1					1
	2002									1				1
Chukotka	2003								1					1
	2004									1				1
	2005								9	1				10
	2006			3										3
	UK												2	2
	Total			3	2			1	11	4			2	23
	2005			1										1
Dim ed	Total			1										1
Sub-total		0	0	0	4	2	0	0	0	1	11	4	0	24
Okhotsk	1995								13					13
	1996								11					11
	Total								13	11				24

Table 2: Distribution of bowhead mtDNA haplotypes among different localities.

Hap	North Slope						St. Lawrence Is.					
	Barrow		Kaktovk	Nuqsut	Wainwright	Pt. Hope	Savoonga	Gambel	Chukotk	Dismal	Ed0	khotsuk
	Spring	Fall										Sea
1	3	6	0	0	0	0	1	0	0	0	0	0
2	0	5	0	0	0	0	0	1	1	0	0	0
3	3	1	0	0	0	0	0	1	2	0	0	0
4	3	2	0	0	0	0	0	0	1	0	0	0
5	9	5	0	0	0	0	2	1	2	1	0	0
6	0	0	0	0	1	0	0	0	0	0	0	0
7	3	2	1	0	0	0	1	1	0	0	0	0
8	1	1	0	0	0	0	0	0	0	0	0	0
9	2	1	0	1	0	1	0	0	1	0	0	0
10	1	2	0	0	0	0	0	0	0	0	0	0
11	0	1	0	0	0	0	0	0	0	0	0	0
12	0	1	0	0	0	0	0	0	0	0	0	0
13	1	1	0	0	0	0	0	0	1	0	0	0
14	1	0	0	0	0	0	0	0	0	0	0	0
15	2	3	1	0	0	0	0	1	0	0	0	0
16	1	0	0	0	0	0	0	0	0	0	0	0
17	1	2	0	0	0	0	0	0	0	0	0	0
18	3	1	0	0	0	0	0	0	0	0	0	0
19	1	2	0	0	0	0	0	0	0	0	0	0
20	2	4	0	2	1	1	0	0	0	0	0	0
21	0	1	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	2	0	0	0	0
23	7	11	1	0	1	2	1	3	3	0	0	0
24	1	2	0	0	0	0	0	0	1	0	0	0
25	1	1	0	0	0	0	0	0	0	0	0	0
26	2	0	0	0	0	0	0	0	0	0	0	0
27	1	1	1	0	0	0	0	0	0	0	0	0
28	3	4	0	0	1	0	0	3	0	0	0	0
29	0	2	0	0	0	0	0	1	0	0	0	0
30	0	0	0	0	0	0	0	1	0	0	0	0
31	3	3	0	0	0	1	0	2	0	0	0	0
32	3	1	0	0	1	0	1	1	0	0	0	0
33	0	0	1	0	0	0	0	0	0	0	0	0
34	0	1	0	0	0	0	2	0	0	0	0	0
35	0	1	0	0	0	0	0	0	0	0	0	0
36	1	1	1	0	0	0	0	0	1	0	0	0
37	1	0	0	0	0	0	0	0	0	0	0	0
38	0	1	0	0	0	0	0	0	0	0	0	0
39	1	2	1	0	0	0	0	0	0	0	0	0
40	0	0	1	0	0	0	0	0	0	0	0	0
41	2	1	0	0	1	0	0	0	0	0	0	0
42	34	29	7	0	1	0	6	10	5	0	13	0
43	3	0	0	0	0	0	2	0	1	0	0	7
44	0	1	0	0	0	0	0	0	0	0	0	0
45	2	1	0	0	0	0	0	3	0	0	0	0
46	3	5	2	0	0	0	0	0	1	0	0	0
47	0	2	0	0	0	1	1	0	0	0	0	0
48	0	1	0	0	0	0	0	0	0	0	0	0
49	1	1	0	0	0	0	0	0	0	0	0	0
50	0	2	0	0	0	0	0	2	1	0	1	0
51	0	3	0	1	0	0	0	2	0	0	0	0
52	0	0	0	0	0	0	0	1	0	0	0	0
53	1	2	0	0	0	0	0	0	0	0	0	0
54	2	0	0	0	0	0	0	0	0	0	0	0
55	0	3	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	1	0	0	0
57	0	1	0	0	0	0	1	0	0	0	0	0
58	1	2	0	0	1	0	0	1	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0
60	1	0	0	0	0	0	0	0	0	0	0	0
61	8	2	0	0	0	0	0	0	1	0	0	0
62	1	0	0	0	0	0	0	0	0	0	0	0
63	1	0	0	0	0	1	0	0	0	0	0	0
64	3	3	0	0	0	0	2	0	0	0	3	0
65	0	1	0	0	0	0	0	0	0	0	0	0
66	0	1	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	1	0	0	0	0	0	0	0
	124	133	17	4	9	7	20	37	23	1	24	0

Table 3: Nucleotide diversity in bowhead whale by locality and stock (in parenthesis is the standard error).

Locality	Nucleotide diversity
Barrow Spring, n=124	0.0106 (0.0009)
Barrow Fall, n=133	0.0120 (0.0009)
Kaktovik, n=17	0.0087 (0.0023)
Nuqsut, n=4	0.0143 (0.0046)
Wainwright, n=9	0.0158 (0.0027)
Pt. Hope, n=7	0.0122 (0.0028)
Savoonga, n=20	0.0113 (0.0022)
Gambell, n=37	0.0128 (0.0015)
Chukotka+Diomed, n=24	0.0104 (0.0020)
Okhotsk Sea, n=24	0.0081 (0.0025)

Table 4: Results of the heterogeneity test for temporal variation at **St. Lawrence Island**. In parenthesis are the sample sizes.

	Chi-square p-value	Fst	P value for Fst
April+May(13)/Nov-Feb(11)	0.1690	0.054	0.0659

Table 5: Results of the heterogeneity test for temporal variation at **North Slope** for two periods: 'Early Years' (1983-2000) and 'Late Years' (2001-2006). In parenthesis are the sample sizes.

	Chi-square p-value	Fst	P value for Fst
'Early Years' April-June(73)/August-October(78)	0.2801	0.002	0.2359
'Late Years' April-June(67)/August-October(76)	0.7564	-0.004	0.8171
'Total Years' April-June(140)/August-October(154)	0.4170	-0.001	0.6426

Table 6: Results of the heterogeneity test for temporal variation at **Barrow** for two periods: 'Early Years' (1983-2000) and 'Late Years' (2001-2006). In parenthesis are the sample sizes.

	Chi-square p-value	Fst	P value for Fst
'Early Years' April-June(70)/August-October(72)	0.2454	0.002	0.2586
'Late Years' April-June(54)/August-October(61)	0.9563	-0.006	0.9376
'Total Years' April-June(124)/August-October(133)	0.5480	0.000	0.3474

Table 7: Results of the heterogeneity test for spatial genetic variation among four localities: Barrow, St. Lawrence Island, Chukotka and Okhotsk Sea. Above diagonal chi-square p value; below diagonal Fst and Fst p value. In parenthesis are the sample sizes.

	Barrow (257)	St. Lawrence Is. (57)	Chukotka (24)	Okhotsk Sea(24)
Barrow	-	0.5200	0.8795	0.0570
St. Lawrence Is.	-0.0023 p=0.7495	-	0.2259	0.0864
Chukotka	-0.0067 p=0.7941	-0.0029 p=0.5147	-	0.0008
Okhotsk Sea	0.0825 p=0.0000	0.0681 p=0.0000	0.1073 p=0.0000	-

Appendix: Number of samples of bowhead whale used in the analysis by LeDuc *et al.* (2007). Grey color indicates differences in data used by these authors regarding those used in the present study.

Strata	Village	Year	Month												UK	Total		
			1	2	3	4	5	6	7	8	9	10	11	12				
	Barrow	1983					1										1	
		1984					3											3
		1986				2	3											5
		1987					1											1
		1988									1							1
		1989					2						3					5
		1990					2						3					5
		1992					2			1	8	1						12
		1993				6	7											13
		1995					3	1			2	2						8
		1996				1	4				18							23
		1997					8	1		1	14	5						29
		1998					9				1	1						11
		1999				1	8					6						15
		2000				1	4				4	2						11
		2001				1	16					4						21
		2002					3				1	18						22
		2003				1	8	1				5						15
		2004				2	3	1			9	6						21
		2005				2	13				2	16						33
	2006					4											4	
	Total				17	104	4		2	60	72						259	
	Wainwright	1981				2											2	
		1984					1											1
		2003				1												1
		2005				2	2											4
		2006					1											1
	Total				3	6											9	
	Kaktovik	1986								2							2	
		1990								1								1
		2000									3							3
		2002									3							3
		2003									2							2
		2004									3							3
	2005									3							3	
	Total									17							17	
	Nuiqsut	1992								1							1	
		2004								3								3
		2005								1								1
	Total									5							5	
	PtHope	2003				3											3	
		2005				1	5											6
	Total				4	5											9	
	Sub-total		0	0	0	24	115	4	0	2	82	72	0	0	0		299	

Appendix: continued

	1984			1									1	
	1989				1								1	
	1996			2									2	
	2001								1				1	
Savoonga	2002									3			3	
	2003									2			2	
	2005	1		3					3				7	
	UK											3	3	
	Total	1		6	1				4	5		3	20	
	1996			1	1								2	
	2002									3			3	
	2003			2									2	
Gambel	2004									1			1	
	2005	1		1									2	
	UK											28	28	
	Total	1		3	1					4		28	38	
	Sub-total	2	0	0	9	2	0	0	0	0	4	9	31	
Chukotka	1998				2								2	
	1999									1			1	
	2000							1					1	
	2001								1				1	
	2002									1			1	
	2003								1				1	
	2004									1			1	
	2005								9	1			10	
	2006			3									3	
	UK											1	1	
	Total			3	2			1	11	4		1	22	
Grand total		2	0	0	12	4	0	0	0	1	11	8	9	32
														80