

ALASKA BELUGA WHALE COMMITTEE
REPORT 02-4

Beluga Whale (*Delphinapterus leucas*) Genetic Studies, 2001 - 2002

Prepared by

Greg O'Corry-Crowe

Southwest Fisheries Science Service
National Marine Fisheries Service
8604 La Jolla Shores Drive
La Jolla, CA 92037

Assisted by

Marc Basterretche

Southwest Fisheries Science Service
National Marine Fisheries Service
8604 La Jolla Shores Drive
La Jolla, CA 92037

Submitted to

The Alaska Beluga Whale Committee

November 19, 2002

INTRODUCTION

Molecular genetic studies on beluga whales, *Delphinapterus leucas*, in the year 2001-2002 consisted primarily of continuing the analysis of stock structure and dispersal patterns in this species throughout Alaska and across the Bering-Chukchi-Beaufort Seas region, through the examination of genetic variation in mitochondrial DNA (mtDNA) and eight microsatellite markers.

Emphasis in the laboratory was placed on mtDNA analysis of tissue samples collected at a number of sites in Norton Sound, Kotzebue Sound and Bristol Bay over the past few years as well as samples from Point Lay and a number of other locations throughout Alaska. We also analyzed a biopsy sample from a whale in Yakutat Bay, the first sample ever collected from belugas in this little known and, as yet, unstudied group of whales (see separate report on Yakutat Bay study). We also initiated a new study to determine if we can extract sufficient amounts of good quality DNA from beluga teeth in order to increase our sample coverage and hopefully begin to look back at what beluga whale population structure and movement patterns were in the past.

Data analysis concentrated on analyzing the new mtDNA data from Norton Sound and Kotzebue Sound in order to determine the relationship between whales in these areas and all other areas. We also completed an extensive analysis of the microsatellite data, which further resolved these relationships and gave us new insights into beluga whale dispersal patterns. This report summarizes the progress on mtDNA analysis of beluga whales during the 12 month period; November 2001 to November 2002. Materials and Methods are the same as in previous reports and in O’Corry-Crowe et al. (1997, 2002). The microsatellite analysis is currently being written up for publication and a detailed report on this topic will be presented next year.

RESULTS AND DISCUSSION

A total of 85 samples were successfully analyzed for variation along 410 base pairs of the mtDNA’s control region and adjacent proline gene (Table 1). This involved extracting the DNA from tissue samples sent to us by hunters and biologists, then amplifying and sequencing the 410bp region we are interested in. Finally, the mtDNA sequence of each individual is scored for its haplotype, or maternal ‘last name’. A further 40 samples that were processed last year were scored for their mtDNA haplotype. When added to our existing sample set, these new samples bring the total number of whales analyzed for mtDNA variation in Cook Inlet, and the Bering, Chukchi and Beaufort Seas to 813. If newborns, fetuses and duplicates are added, the total increases to 832. A further 20 samples have also been analyzed from the Okhotsk Sea.

1. The Five Stocks in Alaska

Samples were added to all five of the currently recognized stocks that occur in Alaskan waters; Cook Inlet, Bristol Bay, the Eastern Bering Sea (Norton Sound-Yukon delta), the Eastern Chukchi Sea (Kasegaluk Lagoon and Point Lay), and the Beaufort Sea (Point Hope-Kivalina in Alaska and the Mackenzie Delta-Amundsen Gulf region in Canada). The increased sample set confirmed earlier findings that these five stocks are genetically and demographically distinct (O’Corry-Crowe et al., 1997, 2002). What this means is that few animals emigrate from one stock and immigrate to another stock, indicating that if one stock became depleted or went extinct, it is highly unlikely that it would recover due to immigration or be re-colonized by belugas from other stocks.

Table 1. Summary of the number of samples that were analyzed for mtDNA variation in 2002, and the total number analyzed in the Gulf of Alaska, the Bering Sea, the Chukchi Sea and the Beaufort Sea to date.

Location	No. of sampling sites	No. of Samples Analyzed in 2002	Total no. Analyzed
Yakutat	1	1	1
Cook Inlet	23	2	92
Bristol Bay	12	9	42
Yukon Delta	3	3	23
Norton Sound	8	29	169
Little Diomede	1	1	3
Kotzebue Sound	7	9	29
Point Hope	2	2	39
Point Lay	2	24	291
Wainwright	1	2	2
Barrow	2	3	13
Mackenzie-Amundsen	12	0	101
eastern Chukotka	3	0	8
total	77	85	813

2. Detailed Stock Structure in western Alaska

As well as confirming the genetic differences between the five current stocks in Alaskan waters, the increased sample size has enabled us to investigate the genetic relationships among different stocks and groupings of beluga whales in the Bering-Chukchi-Beaufort Sea region in more detail.

A. Norton Sound v. Yukon delta

A total of 23 animals have now been analyzed from the Yukon delta region (Emmonak Alakanuk and Hooper Bay). Apart from a new haplotype that was discovered in one of two Alakanuk samples, only haplotypes that are characteristic of Norton Sound have been found in the Yukon and no significant differences in haplotype frequencies between the two regions have been detected to date. Results, however, are preliminary and more samples from the Yukon are necessary.

B. Norton Sound, Spring v. Fall

Last year we reported on some preliminary findings from an analysis of genetic relationships among whales taken in different seasons and at different locations within Norton Sound. We showed that beluga whales taken in the spring might be genetically different to whales taken in the Fall, but stressed that we had not been able to show statistically significant differences between the two seasons. Furthermore, we reported that a detailed breakdown of the data by year, revealed that the differences observed could be attributed to a single year and location. Whales caught in the spring (May - June) in northern Norton Sound (Elim and Cape Nome) in 1996 possessed a number of mtDNA haplotypes never recorded before, or since, in Norton Sound. These 'unusual' haplotypes, however, have been found elsewhere in Alaska. Two of the haplotypes, Hap#9 and Hap#14 are representative of the Beaufort Sea stock, where they have been recorded both at Point Hope and the Mackenzie delta, while Hap#2 is characteristic of the Eastern Chukchi Stock but has also been recorded in the Beaufort Sea and Cook Inlet stocks. Since then, we have analyzed 29 more samples from Norton Sound, including 9 more collected in Norton Bay (Elim, Shaktoolik) in 1996. Two of these were from the spring hunt, and both possessed Hap#9! The remaining 7 were from the fall hunt (September) and all possessed Hap#5, the haplotype most characteristic of Norton Sound.

C. Kotzebue Sound

Last year we reported on a an analysis (now published : O'Corry-Crowe et al., 2002) of samples collected from Kotzebue Sound, the majority of which were collected in one year, 1996. In that year an unusually large numbers of belugas entered the Sound from early to mid June, and a comparison of the 16 samples collected that year to Kasegaluk Lagoon whales from the same year revealed that these two summering areas were genetically different., suggesting that the beluga whales that enter Kotzebue Sound in summer may not be part of the Eastern Chukchi Sea stock. It was also noted that 1996 was the same year that the atypical sample set in Norton Sound (see B. above) was collected and we speculated as to the relationships between these two sets of samples and whether they represented an unusual event that occurred in 1996 or a long established pattern.

In the past 12 months we have analyzed a further 9 samples from Kotzebue Sound that were collected over 5 years from 1997 to 2002. These samples seem to support our earlier contention that Kotzebue is separate from Kasegaluk Lagoon (Point Lay). To further resolve these issues, more sampling is required and a detailed analyses of yearly ice conditions should be completed. Satellite telemetry of belugas in this region should also be conducted if possible.

D. Yakutat Bay

Records of beluga whales in Yakutat Bay are rare and typically consist of 1-5, occasionally 10 or more, animals seen from the air or from shore. The greatest number reported to date was a sighting of 21 whales in May, 1976 (Calkins and Pitcher, 1977). In a recent review of documented beluga sightings in the Gulf of Alaska, belugas were recorded in Yakutat Bay on 8 occasions between 1976 and 2000 (Laidre et al., 2000). Interestingly, these sightings occurred across all four seasons and suggest a preference for the upper reaches of Yakutat Bay and Disenchantment Bay. The paucity of documented sightings has been interpreted as evidence that the beluga whales in Yakutat Bay are not a resident population but more likely occasional visitors from Cook Inlet (Calkins, 1983; Hubbard et al. 1999; Laidre et al., 2000).

Yakutat Bay is a large, relatively deep glacial bay 640 km southeast of Cook Inlet, the location of the nearest beluga whale population. The Bay and associated Disenchantment Bay

are approximately 50 km long in total, are fed by a number of active glaciers and are connected to two long, narrow fiords, Russell Fiord and Nunatak Fiord, atypical habitat for beluga whales in the western Nearctic.

In early May of this year, a group of 4 beluga whales, three white animals and one small, dark grey animal, were seen in open water in upper Disenchantment Bay during a survey of harbour seals on glacial ice by the National Marine Mammal Laboratory's (NMML) Polar Ecosystems Program. Regular aerial surveys and a number of boat-based surveys revealed that the whales used a small ice-free bay or cove between the Hubbard and Turner Glaciers at the head of Disenchantment Bay for a period of two weeks after the initial sighting on May 7 (J. Jansen and B. Adams, Jr., pers. comm.). Following discussions with NMML, AKR, and SWFSC, it was decided to attempt to biopsy a number of these whales for genetic analysis. A number of attempts to get a biopsy from these whales were made by NMML and Department of Agriculture, Forest Service (USDAFS) biologists from kayaks on May 18th. No samples were collected. Photographs and video were taken of the whales during the biopsy effort and on the earlier surveys. Apart from a sighting of a single whale on May 23, subsequent surveys (between May 24 and June 9) failed to locate beluga whales in this well-surveyed zone and suggested that the whales may have dispersed from this region.

In September, Bill Lucey of the US Forest Service, assisted by Bert Adams Jr. and Ray Sensmier, reported sightings of belugas in Disenchantment Bay. Subsequent surveys revealed 6-7 animals swimming in a group. A skin sample was collected from one whale using an air rifle and biopsy dart deployed from an inflatable kayak. We analyzed this sample for mtDNA and found that the individual possessed Hap#2. This finding tells us little as yet about the relationship of the Yakutat whales to other stocks but it is significant in that it has demonstrated that co-ordination with the local community and among government agencies was successful in surveying these elusive animals, launching a field project, and getting a genetics sample. Our hope is to continue this research in the coming years.

3. Extraction and Analysis of DNA from Beluga Whale Teeth

Recent advances in the field of extracting DNA from hard tissues such as bone and teeth potentially opens the door to answering a whole new set of questions about population structure and dispersal patterns that we could not address before. Before 1988 few of the tissue samples collected from beluga whales in Alaska were preserved in a way that we could extract and analyze their DNA. Soft tissues such as skin, when collected were usually stored in formalin. We have done some experiments on extracting DNA from such tissues but have had only limited success (reported on at a previous meeting). For decades, teeth have routinely been collected by biologists in order to determine the age of an animal by counting the growth rings in the cementum.

We initiated a project this year to extract DNA from beluga teeth collected from Elephant Point, Kotzebue Sound in the early 1980s and attempt to analyze the DNA for variation in mtDNA. The method of DNA extraction, amplification, and sequencing is more complicated and time-consuming than the usual method for soft tissues and begins with drilling the tooth in order to powder the enamel, dentine and cementum. Our early findings are mixed but encouraging. We have successfully extracted DNA from a number of teeth but because the amount of DNA is so low, there is a greater danger of amplifying background DNA. We have managed to sequence a

fraction of the samples, including a few whales where we also had soft tissue. These animals acted as positive controls to determine if we were sequencing the particular whale's DNA or background DNA. In two cases we got good sequence and were able to score the animal for its mtDNA haplotype. One was from Elephant Point and it possessed Hap# 5. The other was from Point Lay and it possessed Hap#16.

More work needs to be done on perfecting the laboratory methods, but these initial results are promising. Optimizing the analysis of DNA from teeth will enable us to analyze samples from areas where few skin tissue samples exist.

ACKNOWLEDGMENTS

Funding for this research was provided by the Alaska Beluga Whale Committee (ABWC) and the U.S. National Marine Fisheries Service (NMFS). We are indebted to the native hunters of coastal Alaska and northwest Canada who took the time to collect many of the samples used in this report. Thanks to Marc Basterretche for his expertise in the lab. Thanks to Robert Suydam, Kathy Frost, and Barbara Mahoney for co-ordinating much of the sample collection. We're grateful to Bill Lucey for all his efforts with the Yakutat whales this year and for getting the shot! Thanks are also due to the North Slope Borough for administrative and general support.

REFERENCES

- Calkins, D.G. and Pitcher, K.W. 1977. Unusual sightings of marine mammals in the Gulf of Alaska. Abstract, Proceedings of the 2nd Conference of the Biology of Marine Mammals, San Deigo, CA.
- Calkins, D.G. 1983. Marine Mammals of lower Cook Inlet and the potential impact from outer continental shelf oil and gas exploration, development and transport. U.S. Dept. of Commer., NOAA, OCSEAP Final Rep. **20**: 171-265.
- Hubbard, J.D., Hansen, D.J., and Mahoney, B.A. 1999. Winter sighting of beluga whales (*Delphinapterus leucas*) in Yakutat-Disenchantment Bay, Alaska. *Arctic*, **52** (4): 411-412.
- Laidre, K.L., Shelden, K.E.W., Rugh, D.J., and Mahoney, B.A. 2000. Beluga, *Delphinapterus leucas*, distribution and survey effort in the Gulf of Alaska. *Marine Fisheries Review*, **62** (3):27-36.
- O'Corry-Crowe GM, Suydam RS, Rosenberg A et al. (1997) Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. *Molecular Ecology*, **6**, 955-970.
- O'Corry-Crowe GM, Dizon AE, Suydam RS, Lowry LF. (2002). Molecular genetic studies of population structure and movement patterns in a migratory species: the beluga whales (*Delphinapterus leucas*).