The Marine Mammal Protection Act of 1972 requires the identification of management stocks of all species of marine mammals in US waters, including beluga whales. The Alaska Beluga Whale Committee (ABWC) signed a cooperative agreement with the National Marine Fisheries Service (NMFS) in 1999 to co-manage beluga whale stocks in the Bering, Chukchi and Beaufort Seas. As early as 1992, however, NMFS and the ABWC initiated a cooperative research project to identify management stocks and investigate the movement and social behavior of beluga whales using modern molecular genetic techniques.

Overview - Beluga whale co-management and the role of molecular genetics

Beluga whales are difficult marine mammals to study. They spend much of their time under water where they can’t be seen. They live in northern arctic and subarctic waters where few people go, and they are often found in the sea ice far from shore.

Northern Native peoples have learned many things about the lives of belugas through thousands of years of observation. Because belugas are such an important subsistence resource for many coastal communities, the hunters have learned many things about beluga whale behavior, migration routes and their annual return to specific coastal sites. In recent decades, scientific studies of belugas have added to the traditional ecological knowledge of Alaska Natives. Much of this cooperative research has been directed towards better co-management of this important subsistence species.

The goal of management is to maintain healthy populations of beluga whales while making sure that belugas can be harvested by Alaska Natives in coastal communities in a sustainable way. At present, important management issues include stock identity, population abundance and health, and the effects of human activities (such as disturbance, harvest and contaminants) on belugas. Future concerns will likely include questions about habitat requirements and climate change. The ABWC was a pioneer in recognizing how several new scientific approaches could be valuable for studying and managing beluga whales. This issue of The Alaska Beluga Whale Newsletter focuses on the story of one such approach: molecular genetics.

Twelve years ago, when we started our research program on beluga whales, the use of molecular genetics for studying marine mammals was still very new. With the help and collaboration of the ABWC, the National Marine Fisheries Service (NMFS), and other organizations and agencies throughout the Arctic, we have made huge progress towards understanding and managing this species by using of the most modern molecular techniques. The first human DNA ‘fingerprint’ was made in 1985, revolutionizing the study of human relatedness, disease investigation and forensic (law-related) science. Less than 10 years later we were fingerprinting beluga whales. DNA was first extracted from ancient bones and tissues in the early 1980s. Today, we routinely extract and analyze DNA from beluga teeth, bones and dried tissue. In the article on Molecular genetics and its use in wildlife management, we’ll see how molecular genetics can be used to study and help us manage wildlife, including beluga whales. This is followed by an article on Stock structure of beluga whales in Alaska, northwest Canada and Russia, which
Molecular genetics and its use in wildlife management

Many things go into making up who we are, but the genetic code is the blueprint that pre-determines most of the physical characteristics and many of the behavioral traits of an individual. This includes everything from eye and hair color to how we interact with the world around us. Each characteristic is coded by one or a few genes (pieces of DNA), and the entire code is contained within our DNA. Because of this, we can learn much about an individual by looking at his or her DNA (for example, what color eyes they will have or what genetic diseases he or she may be susceptible to).

We can also simply examine parts of the DNA that allow us to tell one individual from another, a process called DNA ‘fingerprinting’. The fact that we inherit our DNA from our parents also means that we can tell how individuals are related by comparing their DNA profiles. This process is called kinship analysis. In fact, we can reconstruct entire family trees over several generations. We can also find the relationships among different families or social groups, and even among populations and separate species. The less related two individuals or two populations are from each other, the more different their DNA will be. For example, two Inupiat Eskimos will share a lot more characteristics than say, an Irishman and an Inupiat!
All of these techniques have been useful in the study and management of wildlife, and we have used this molecular ecological approach to study beluga whales. Molecular genetics allows us to look at specific genes or regions of the DNA in order to address specific questions.

The first job is collecting enough DNA for study. Fortunately, a copy of a beluga’s DNA is in every cell in its body. We need only a small tissue or blood sample to provide the entire genetic blueprint. We also need samples from enough individual whales for our research to be useful for co-management. We have been extremely fortunate in getting enough samples. Native hunters and biologists from many areas have collected skin samples from hunted, stranded or live whales. Over the years we have amassed a truly exceptional collection of samples from over 1,300 whales at 85 sites across the species’ entire range, including Alaska, Canada, Russia, Greenland and Norway.

Our research has focused on identifying separate management stocks of beluga whales. We do this by determining how the groups of belugas are related, how they disperse, and by mapping their migration routes. We have also estimated levels of genetic diversity and investigated pod and herd structure. More recently, we have developed methods to study the DNA in beluga teeth and bone and have begun to compare our genetic findings with data from satellite telemetry, TEK and surveys.

Stock structure of beluga whales in Alaska, northwest Canada and Russia

The main focus of our beluga genetics research since the beginning has been learning about stock identity. This has required the help of many energetic and enthusiastic technicians, including Aviva Rosenburg, Monica DeAngelis, Lauren Hansen and Marc Basterretche, and most recently Carolina Bonin.

Our studies with mtDNA (see Box ) have found many different maternal lineages (haplotypes) in the belugas that occur in Cook Inlet, and the Bering, Chukchi and Beaufort Seas. We have found that the frequency of the different haplotypes differs greatly among geographically separate summering grounds. This tells us that female belugas tend to return to the same summering grounds year after year, generation after generation, and rarely disperse from one summering group to another. This tendency indicates that the whales of a particular summering ground interact very rarely with whales from another ground and should thus be managed as separate stocks.

Beluga whales that occur in Cook Inlet were the most distinct, indicating that this population of whales is isolated from all others. Summering concentrations of whales in Bristol Bay, Norton Sound, the Chukchi Sea coast of Alaska and the Mackenzie Delta in Canada were also all very different from each other. Haplotype frequencies of whales that migrate past Point Hope in spring were very similar to that of whales that summer in the Mackenzie, suggesting that these two groups are part of the same stock.

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Figure. The frequency of different mtDNA haplotypes in Cook Inlet, four summering grounds in the Bering, Chukchi and Beaufort Seas and one spring migration location, Point Hope. Each haplotype is represented by a different color.

As hunters have sent us more samples, we’ve also started looking at relationships among other groupings. This includes belugas in the Yukon Delta, Barrow, Little Diomede Island and Chukotka in Russia. Preliminary findings indicate that Yukon belugas are closely related to belugas that occupy Norton Sound from spring to fall. Belugas that occur off Barrow in summer are part of the Beaufort Stock, the same stock that occurs at Point Hope and in the Mackenzie Delta.
Samples collected in the Bering Strait region suggest that whales migrating north past Sereniki at the tip of the Chukotka peninsula and near the Diomede Islands in spring are also part of the Beaufort Sea stock. Thus, we have evidence of at least one stock that is shared by three countries, Alaska (US), Canada, and Russia. Furthermore, we have been able to piece together the migration path of this stock from their likely wintering ground southwest of the Bering Strait north through the Strait into the eastern Chukchi Sea, where they pass close to Point Hope. This stock continues northeast past Barrow and arrives in the Mackenzie Delta by July.

Currently, we are focusing our attention on the relationship between belugas that visit Kotzebue Sound in summer and other belugas in the Bering-Chukchi-Beaufort region. We have also just begun a study to determine the origins of a small group of beluga whales that have been observed in Yakutat Bay in the Gulf of Alaska each spring and summer.

**Tracking changes over time – the beluga whales of Point Lay.**

When we started our genetic studies on beluga whales, the big question was stock structure. Were the whales at one location part of the same stock as whales at another location? For example, were the whales seen in Norton Sound each spring and summer part of the same stock that swims north along the Chukchi Sea coast past Point Lay each June and July? By using mtDNA, we were able to confirm the long held belief of local communities that these two summering groups were indeed separate sub-populations that should be managed as separate stocks.

Differences among human communities develop over long periods of relative isolation (remember the Inupiat Eskimo and the Irishman!). We thought that the genetic differences among the various beluga stocks probably also took a long time to develop. This would involve generations of belugas returning with their young to the place of their birth each year. A nagging question remained, however. Could this picture of stock discreteness change over a relatively short period of time? No biological system stays the same forever. Beluga whales may tend to do the same thing year after year, even generation after generation, but they must also be able to change their behavior if necessary, for example if their environment changed. It is conceivable that such a change could happen fairly quickly.
Mitochondrial DNA

There are many potential genetic markers that could be used to study population structure and breeding behavior in wildlife species. However, mitochondrial DNA (mtDNA for short) stands out as the most useful marker in studies of stock identity: A genetic marker needs to be (A) variable and (B) heritable (that is, passed from one generation to the next). Mitochondrial DNA is highly variable, with each variant called a haplotype. It is unusual because unlike most other genetic markers, mtDNA is inherited only through the maternal line. In other words, only mothers pass their mtDNA haplotype to their offspring. In turn, daughters pass that same haplotype to their offspring. Thus, we can potentially trace entire family pedigrees of belugas back several generations by looking at the mtDNA. This ‘maternal’ inheritance is similar to a number of Alaskan Native cultures where relationships are traced through the mother’s line (e.g., Inupiat, Aleut and Tlingit society).

MtDNA haplotypes can be looked at as family or last names in human societies, except that in western societies the inheritance of names is paternal (from the father). We can learn a lot about human cultures by documenting the frequency and location of family names. For example, we can compare last names in two villages in Alaska. If we find that very few family names are shared among both communities, we can conclude that there has been very little intermarriage between the two, at least in terms of men moving from their village of birth. If we compare a large city to a number of villages, we may find many more family names in the city than in any one village. This is due to migration of people from the smaller communities to the larger city. And so, we’ve learned a lot about breeding behavior and movements by simply looking at the distribution of family names. Similarly, we can track breeding behavior and movements of female belugas by mapping the distribution of mtDNA haplotypes.

Clearly we needed to tackle this question to better understand beluga stocks. But, it was easier said than done! We felt we would need to collect tissue samples year after year if we were going to learn if, and how quickly, belugas might change their migration patterns. There are few locations where it is possible to conduct such a study. Point Lay on the Chukchi Sea coast is one of them. Thanks to the efforts of the Point Lay community, the North Slope Borough Department of Wildlife Management, the Alaska Department of Fish and Game and the ABWC, beluga whales have been sampled and studied at Point Lay since the early 1980s.

From the very beginning, collecting samples for genetics was a priority. Seventeen years later, our analysis shows that the genetic composition (variation in mtDNA) of the beluga whales migrating past Point Lay each summer has changed little from 1987 to 2003. We can thus conclude that the same sub-population or herds of beluga whales have migrated along the Chukchi Sea coast each summer of our study. We will continue to monitor this and other groups of belugas throughout the arctic year after year.

Ancient DNA – old bones reveal secrets from the past

Our studies up to now have required collecting and preserving blood or soft tissue (such as skin or muscle) from live or recently hunted or stranded whales. When the soft tissues decompose, it is much more difficult to extract good quality DNA. For this reason, it has been necessary to either freeze or preserve these tissue samples in DMSO and salt immediately after the sample has been collected. The preservative does two things. It dehydrates the cells thus preventing hydrolytic (water) damage of the DNA and it protects the DNA from microbial breakdown and oxidative (air) damage. The limitation of all this has basically been that we’ve been unable to get DNA out of tissues that were not preserved immediately after collection. That is, until recently. Breakthroughs in molecular biology are now making it possible to get DNA out of dried tissues, bones and teeth, and in some cases extremely old tissue and bones. For example, scientists have successfully extracted DNA from ancient Egyptian mummies, primarily because the tissue was kept dry and...
cold deep inside tombs, in some cases for thousands of years! Using the same methods we were successful in getting DNA out of tiny pieces of 20 year-old dried tissue still clinging to beluga whale jaws collected in the 1970s and 1980s. Furthermore, we’ve been able to analyze the genetic material and use these animals in our analysis of stock structure.

Bone and teeth contain very small amounts of DNA, encased in large amounts of mineral deposits, primarily calcium. This protects the DNA from the damaging effects of water and air, but also makes it very hard for the scientists to find. Recently, a research group in Germany developed methods to successfully extract DNA from the bones of long extinct mammals, including mammoths and Pleistocene horses. Some samples were radiocarbon dated to be over 25,000 years old! We recognized the potential to fill in sample gaps and expand our time series studies if we could use these ‘ancient DNA’ methods to get DNA from beluga teeth and bones. A number of ABWC members including Chairman Ross Schaeffer and Kotzebue delegate Willie Goodwin, had shown interest and encouraged us to see if it would work.

In September 2003, we initiated a study with ABWC funding to apply, and develop if necessary, ‘ancient DNA’ methods to beluga whales. Considering the very small amounts of DNA involved, a major concern in this kind of research is excluding any possibility of contamination from our normal studies, even from the tiny amounts of DNA that are present in the air. We, therefore, helped set up a new lab, separate from our current lab. This lab has state-of-the-art air filters and equipment to ensure a sterile working environment as much as possible. Technicians must wear special clothing to prevent contamination of the samples. Two technicians, Matthias Meyer visiting from the German lab that pioneered much of the early work, and Amy Frey from Southwest Fisheries, have worked on this project for the past year, perfecting methods to get DNA from beluga whale teeth and bone. Their work resulted in the successful extraction and analysis of DNA from the teeth of over 30 whales collected in Kotzebue Sound in 1982 and 1983.

Over the past year we have continued to develop, test and perfect methods for getting DNA out of unusual material, such as dried muscle, bone and teeth. Recently, we attempted to extract DNA from truly ancient material - mammoth tooth and bison bone from the North Slope, and seal and pilot whale bones from an archaeological dig on the Aleutian Islands. The seal and whale samples have been provisionally aged to be about 3,000 years old! To date, we have sequenced DNA from most of these ancient bones and teeth, including the mammoth. The mammoth was estimated to be 39,000 years old!

The remarkable early success of this research opens the door to a host of exciting and important scientific and management questions. For the first time we may be able to look back into the distant past and study the beluga whale populations of another time. We may finally be able to determine how the beluga stocks of today have changed over time. The ability to analyze DNA from teeth and bones also allows us to fill in some of the sampling gaps in our analysis of stock structure.

Genetic detectives – DNA fingerprinting helps solve mystery

Perhaps the best known application of DNA analysis today is the use of DNA fingerprinting in forensic (legal) investigations. Just as every human has a unique pattern of swirls on the tips of their fingers, or fingerprint, every individual also has a unique genetic code or ‘DNA fingerprint’. In wildlife studies we use this characteristic to identify individual animals. This is a particularly useful tool in the study of beluga whales, as individual whales are often hard to tell apart from other whales in the field.

One of the most remarkable aspects of the migration behavior of beluga whales is the apparent consistency in the route and timing of the migration from year to year. Each summer towards the end of June, beluga whales are typically seen migrating north along the Chukchi Sea coast.
near Point Lay. Each summer a number of belugas are harvested by the local community. As mentioned earlier, we have been able to compare the genetic profile of the hunt across many years and have shown that the same haplotypes are seen in the same abundance each year.

On June 29, 1998, a team of biologists and hunters led by Robert Suydam caught and tagged 5 belugas (just after the annual hunt). The whales were recorded making extensive movements out into the Chukchi and Beaufort Seas before the tag’s signals failed several months later. And that was the last we expected to hear from these animals. On June 30, 1999, exactly a year after the 5 whales were tagged, a beluga was caught in the annual hunt with a curious scar on its dorsal ridge. Could this whale have been one of the ones tagged the year before? Returning with a skin sample to the lab we were able to ‘fingerprint’ the whale and determine that it was indeed one of the big males that we caught and tracked in 1998. This finding showed us just how precise the timing and route of beluga whale migration can be. Despite having covered several thousand miles across three seas, this whale was right back at the exact location where it had been one year earlier.

The private lives of beluga whales as told by the genes

Beluga whales are highly social animals. They are usually seen in groups of a few animals to herds of a few hundred, but often form aggregations of 1,000 or more whales. Belugas are also highly vocal and there are many accounts of belugas interacting together while foraging, resting, and caring for their young. Belugas are often observed to play and there are many stories about their obvious intelligence.

So how is beluga whale society organized? Are herds made up of closely related individuals or of entirely unrelated whales? If families tend to stay together, are they organized along maternal lines, as is the case in some other species such as killer whales and pilot whales?

One way to address some of these questions is to figure out the relatedness among whales by using genetic markers. In most of the cases where we have been able to analyze several animals from a single group or herd, we have found that, except for mothers and their calves, the whales were not closely related. Furthermore, we typically found many different mtDNA lineages (haplotypes) within a single pod or herd, indicating that, unlike killer and pilot whales, pods and entire herds of belugas are not necessarily composed of single families (matrilines).

Mark-recapture studies

In the early days of ecological studies, some bright spark figured out that if you caught and marked (e.g. tagged, branded) a fraction of a population in one year and then returned the following year and caught the same number of animals and checked which fraction had the mark, and were thus recaptured, you could estimate how large the population was without having to see and count every animal in the population. Such mark-recapture studies also allow you to learn other important aspects of a population, including the proportion that are male versus female, what proportion are immature versus mature and what proportion are capable of breeding.

In studies of whales, catching and marking a substantial portion of a population is simply not a viable option. Fortunately, some whales have unique natural markings that can be used as tags. For example, each humpback whale has a unique pattern of light and dark coloration on its tail flukes that allow identification of known individuals. Similarly, Killer whales have distinctive shapes to their dorsal fins and unique markings on their backs. All that is required, therefore, is to photograph these identifying patterns.

Alas, belugas have few such diagnostic features. However, because we can biopsy and get DNA fingerprints from belugas we can now effectively conduct a genetic mark-recapture study. Each individual beluga has a unique DNA fingerprint. The first time we biopsy a beluga and get its DNA fingerprint, it counts as a “mark.” If we biopsy the same beluga again in another year, it will count as a “recapture.” We can then use this information to estimate how big the population is. We can also compare the results of the mark-recapture study with aerial survey results to decide which is the most reliable and cost-effective method. The Bristol Bay Marine Mammal Council, in cooperation with ADF&G, has already started a mark-recapture study in Bristol Bay. We have plans to start a similar study in Cook Inlet.
**Future plans**

Our ability to collect skin samples from free swimming whales by using jab stick, crossbow or air rifle with a specially designed dart, combined with the power of DNA fingerprinting, now make it possible to address many new questions of importance to management. These include estimating population abundance and monitoring individual animals throughout their entire lives. The power to extract genetic information from teeth and bones opens up many new opportunities to fill sampling gaps in key areas like Chukotka and Kotzebue Sound. We can now explore changes in stocks over time by digging up and examining ancient bones and teeth. We also need to start looking at new molecular markers that allow us to investigate many different questions. These include estimating age and studying male dispersal patterns.

**Final comments**

The various studies we have conducted over the past dozen years have answered many questions about the life of the beluga whale. Each study is one piece of a challenging puzzle. Collectively, the research is an exceptional body of work and stands as a testament to the foresight and teamwork of the Alaska Beluga Whale Committee, its members and partners. Much of this work has been published in scientific journals and has contributed to the identification of management stocks. Our collective task now is to put the pieces of the puzzle together and identify those areas that still need to be addressed.

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