SC/66b/BRG/17

Meeting to Discuss the Biology and Genomics of Bowhead Whales

John W. Bickham, J. Craig George, Robert Suydam

INTERNATIONAL WHALING COMMISSION

Meeting to Discuss the Biology and Genomics of Bowhead Whales

JOHN W. BICKHAM[1], J. CRAIG GEORGE[2], ROBERT SUYDAM[2]

[1]Battelle Memorial Institute, Houston, TX
[2]Department of Wildlife Management, North Slope Borough, Barrow, AK

ABSTRACT

On October 14 and 15, 2015, Battelle and the North Slope Borough of Alaska (NSB) held a symposium to examine the role of genomics in bowhead whale conservation and management. The goals of the workshop were to 1) obtain guidance and advice on the future of the bowhead whale genetics and genomics program, 2) to inform the NSB and the Alaska Eskimo Whaling Commission (AEWC) about current applications of genomics research and how advances in this field might benefit the bowhead genomics program, 3) to discuss AEWC's perceived risks to pursuing genomics research on bowheads, and 4) to educate current and potential collaborators regarding the political and social realities of conducting research in an extreme environment related to an endangered species which is the central focus of the native Alaskan's culture and subsistence diet.

INTRODUCTION

The conference (October 14 and 15, 2015) brought together researchers and stakeholders from across the country with representatives of the oil & gas industry, the North Slope Borough, the International Whaling Commission (IWC), and indigenous communities (Figure 1, Appendix 1). The two-day symposium, held at Battelle's headquarters in Columbus, OH, provided participants with opportunities to network and catch up on the latest developments in the field.

Scientists shared updates from their genomic research at the symposium. Additional sessions covered the biology of bowhead whales, an introduction to genomic research methods, and presentations on metagenomics, population genomics and mitochondrial genetics. Each day featured a special lunch presentation. On day one, Harry Brower Jr. of the North Slope Borough discussed the bowhead whale's role and importance in native Eskimo culture. On day two, author Hans Thewissen shared research on whale evolution and fossil documentation from his latest book, *The Walking Whales*. The symposium wrapped up with a panel discussion summarizing lessons learned and next steps for the research community, IWC and North Slope Borough.

Bowhead whales are important to native Alaskan Eskimo communities for both subsistence and ceremonial purposes. They are also protected by national and international conservation laws. Oil & gas developers and other industries operating in the Beaufort and Chukchi Seas must understand bowhead whale populations and migration patterns so that their activities do not negatively impact either whale populations or the Eskimo hunts, both of which are protected by the Marine Mammal Protection Act.

Figure 1. Photograph of the meeting participants.

Genomic research conducted on behalf of IWC, the NSB and the AEWC has helped scientists answer important questions about the size and diversity of the bowhead whale population. Genomics also provides insights into whale evolution, population dynamics, movement patterns and the spread of diseases in populations. The research helps scientists better understand the vulnerabilities of specific bowhead populations and of the species as a whole.

The IWC uses this research to set hunting quotas for Eskimo communities. It is also used to help the oil and gas community understand the risks of different activities and make better decisions to balance conservation and development priorities. The symposium brought stakeholders together to further define the role of genomics in environmental monitoring and conservation and specifically in the bowhead genetics project funded by the North Slope Borough.

Background

Mission Statement of the Department of Wildlife Management, North Slope Borough: *The Department of Wildlife Management facilitates sustainable harvests and monitors populations of fish and wildlife species through research, leadership, and advocacy from local to international levels. The Department diversifies funding opportunities through the submission of grant proposals focusing on subsistence species and issues of the highest interest to North Slope residents. With regard to bowhead whales, one of the main responsibilities of our Department is the bowhead population abundance work and other studies to support sustainable harvest levels.*

Bowheads are central to the Inupiat/Yupik culture. The collective goal of the AEWC and NSB is to maintain healthy populations and encourage applicable research. Said another way, the goal is to conduct studies that are good for the species, good for local communities and good for society in terms of scientific contributions.

The NSB has an interest in genomics work that supports the goals of the DWM mission statement. That is, work that provides information useful to conservation of the resource and in support of efforts to maintain the quota provided by the IWC. The AEWC and NSB has no interest in other potential applications of genetics, such as patenting genes, or the development of

any commercial products directly or indirectly from whales. Notwithstanding that such products might be commercially valuable and even beneficial to society in general, the community must first protect the IWC quota. Accordingly, the bowhead whale hunt must be conducted as a completely non-commercial activity and actions that might be construed as commercial in nature could put the quota at risk. Because the IWC is highly political, the AEWC and NSB take a very conservative approach to the approval of the use of bowhead tissues and other related resources for projects that do not directly serve the mission statement.

With regard to the biological research, the AEWC retains full control of the samples that are sent out for various studies. These samples are essentially the property of the Captain's and AEWC. Through the years, some studies of a very basic nature, for example lung anatomy, have been supported as these have not been seen as a potential path to any commercial activity. But basic genetic and genomics is more problematical due to the common practice of identifying and patenting genes with potential medical application. The hunters have repeatedly said that it's not acceptable for a research group to achieve monetary gain from samples provided by the whaling community, especially when the community does not share in the benefits.

Workshop Goals

The goals of the workshop were to 1) obtain guidance and advice on the future of the bowhead whale genetics and genomics program, 2) to inform the NSB and the Alaska Eskimo Whaling Commission (AEWC) about current applications of genomics research and how advances in this field might benefit the bowhead genomics program, 3) to discuss AEWC's perceived risks to pursuing genomics research on bowheads, and 4) to educate current and potential collaborators regarding the political and social realities of conducting research in an extreme environment related to an endangered species which is the central focus of the native Alaskan's culture and subsistence diet.

It is hoped that by convening this group of experts on genomics, bowhead biology, and related areas of interest, that the group can arrive at recommendations regarding future bowhead genomics studies, including those specifically addressing the mission statement as well as others that might indirectly benefit the community.

Keeping in mind that the overall goal of the NSB genetics program is to support sustainable harvest, good management, and good science, one of the overarching objectives of any proposed study should be: How does the work help the community and bowhead whales?

RESULTS AND DISCUSSION

A diverse group of scientists and stakeholders attended the meeting and the topics discussed represented a broad diversity of topics of relevance to bowhead biology, genetics and genomics, and to the NSB and AEWC. Below we present the abstracts of the presentations given during the technical sessions.

Abstracts of technical presentations

Amy B. Baird. Study of bowhead genetics using single nucleotide polymorphisms (SNPs)

An overview of the Fluidigm method for SNP genotyping was given. The project aims to utilize SNP data to examine stock structure, familial structure, and historical population demography of bowhead whales. Preliminary data from 285 bowhead whale samples genotyped at 96 loci were presented. In particular, SNPs from the X and Y chromosomes were discussed. Currently, data from 6 X chromosome loci and 1 Y chromosome locus have been used to successfully distinguish the sex of samples, as well as yielding X chromosome haplotypes that will be used in future population demography estimates.

Scott Ballinger. Mitochondrial – Nuclear eXchange (MNX) models and Mito-Mendelian Genetics: Relevance to Disease

By virtue of the endosymbiotic origins of the eukaryotic cell, I hypothesize that it is mitochondrial – nuclear genetic interactions that significantly influence cell function and response to stressors. Genetic selection processes for mitochondrial – nuclear interaction are influenced by survival and reproductive success; thus environmental challenges affect the cell/organism based upon their respective mitochondrial and nuclear genetic backgrounds that combined, influence cellular function in cardiovascular, hepatic, endocrine (adipose), and inflammatory (monocytes-macrophage) systems. To facilitate direct comparisons, we have developed the Mitochondrial – Nuclear eXchange (MNX) model that enables unambiguous testing of different mitochondrial and nuclear genetic backgrounds upon cell function and response to stressors. From this research, it is clear that mitochondrial genetic background and function significantly influence cellular response to challenges and thus, are a key etiological factor in the development of cellular dysfunction important in various disease and aging processes. It is the interaction of nuclear and mitochondrial genomes that govern the genetic basis for cell function and disease, which heretofore are commonly thought to be explained by Mendelian genetics. Instead, I propose that cellular adaptation and genetic evolution are governed by "Mito-Mendelian" genetics. This concept is paradigm challenging.

John Bickham. Overview of Bowhead Genetics and Genomics

The bowhead whale is remarkable for its great size, longevity and its importance as a dominant food source in the subsistence diet of Eskimo communities of northern Alaska. This presentation discussed genetic studies conducted in support of the efforts of the Department of Wildlife Management of the North Slope Borough to monitor the population and manage the subsistence harvest. These studies have included analyses of population diversity and population structure, evolutionary history, and most recently the description of the bowhead whale genome and transcriptome. This is only the second species of baleen whale from which a genome sequence has been reported. The genome sequence represents a valuable resource for researchers from a variety of disciplines and was used to develop a panel of nuclear SNP genetic markers that are presently being used for population and evolutionary studies of BCB bowheads.

J. Andrew DeWoody. Population Genomics in the Absence of Dispersal Barriers.

Genome sequencing can identify genetic markers (SNPs) that yield rapid, informative, repeatable, and inexpensive genotypes that can be used to sex and genetically tag individuals. These genetic tags can then be used a) in a DNA mark-recapture framework to estimate census population sizes and changes over time; b) in a population structure analysis to identify distinct gene pools, if any; c) in a relatedness framework to identify close relatives (parents/offspring, siblings, etc.). Finally, these same markers can be used to identify regions of the genome that may be involved in disease resistance, thermoregulation, oxygen binding, etc.

Mike Dickens. Metagenomics

Battelle has over two decades of multidisciplinary (e.g., microbiology, molecular biology, genetics, and bioinformatics) expertise and experience collecting, processing, analyzing, storing and shipping microbial samples and sample extracts from a wide variety of environmental matrices. Battelle's experience includes extraction, purification, and testing for the presence of microbial agents, including next generation sequencing of nucleic acids, from a variety of matrices, including laboratory-cultured organisms, insects, animals, plants, soils and water samples collected from around the world, as well as complex matrices such as sediments contaminated with hydrocarbons. All of Battelle's studies adhere to an established, robust Quality Management System (QMS) in accordance with the ISO 9001:2008 Standard, and where applicable, Good Laboratory Practices (GLP). Examples discussed during the Bowhead Genomics Conference included:

- Microbial metagenomics analysis of oil biodegradation in Alaska and Rhode Island sediments
- Microbial metagenomics analysis of the biodegradation of hydrocarbons following the Deepwater Horizon Accident
- Microbial metagenomics analysis of microbial response(s) following *in situ* oil exposure
- Microbial metagenomics analysis to determine the cause of corrosion in ultra-low sulfur diesel tank systems
- Environmental DNA (eDNA) analysis to detect and type gray whales using sloughed DNA suspended in sea water

Craig George and Robert Suydam. Bowhead Basics: Biology, Management and Whaling

The bowhead whale is a large pagophylic or "ice-loving" member of the Balaenidae which inhabits the ice-associated regions of the Arctic and sub-Arctic seas. Bowheads are born in sub-zero sea water in spring within the ice-lead systems of northwest Alaska - they are the only mysticete that gives birth in Arctic waters, and the only mysticete that spends its entire life in polar regions. Bowheads are an important subsistence species for many coastal native communities in Russia, Alaska and Canada. They may exceed 19 m in body length and 80 metric tons in body mass. The bowhead whale exhibits a number of superlatives among Cetacea. These include: the thickest blubber, greatest longevity, longest baleen, low body core temperatures, and may have the largest head-to-body length ratio of any whale. Maximum ages have been estimated to reach 200 years.

Four stocks are recognized across the arctic. The stocks are considered genetically different; however the Eastern Canadian and Bering Chukchi Beaufort Seas (BCBS) are closely related.

Before whaling reached its peak in the late nineteenth and early twentieth centuries, an estimated 50,000 bowheads swam the circumpolar arctic region. The largest stock was the Spitzbergen stock in the arctic North Atlantic. Commercial exploitation of the species by Europeans began as long ago as 1611 in the eastern Atlantic Arctic, and over the succeeding 200 years, the eastern Arctic stock was reduced from an estimated 30,000 to about 1,000. Similarly, in the western arctic bowheads were severely depleted between 1848-1915 with perhaps only 1,000 animals surviving from, a former population of 20,000 or more. The current BCBS stock size is estimated at about 17,000 animals.

Intensive research began on the BCBS stock of bowheads in 1970s mainly over concerns about the population size and effect of the Native harvest. In addition to the population studies, many other studies were conducted including: anatomy, food habits, acoustics, evolutionary biology, hearing, pathology, genetics, and satellite telemetry.

The population is managed under a quota system by the Alaska Eskimo Whaling Commission which includes of 11 communities, through an agreement with the U.S. National Oceanic and Atmospheric Administration. The US is a member nation within the International Whaling Commission where the overall harvest level is determined by the since.

As of 2015, the quota is 67 strikes or 56 landed per year for the 11 villages. Typically, about 40 to 45 whales have been landed annually over the past 10 years. The quota is based on the nutritional and cultural needs of Alaskan Eskimos as well as on estimates of the size and growth of the Bering-Chukchi-Beaufort Seas stock of bowhead whales. Whales were harvested in 2015 under a six-year block quota that began in 2013.

The subsistence hunt is extremely important to the communities for cultural and nutritional reasons, and typically takes place during spring and autumn as whales migrate between the Bering and Beaufort Seas. Hunters on Saint Lawrence Island in the northern Bering Sea may harvest whales during the winter (i.e., December and January) as well. The bowhead hunt is highly affected by weather, stability of landfast ice, sea ice concentration and dynamics. Hunting success (the number of landed whales) therefore shows considerable annual and regional variation due to these environmental factors.

Geof Givens. Using stock abundance and structure analyses to guide bowhead management.

Dr. Givens spoke about "Using stock abundance and structure analyses to guide bowhead management", presenting background information about bowhead science and management. In the first half of his talk, Givens reviewed global bowhead population structure, explained the subsistence harvest, and discussed IWC investigations into potential stock structure within the Bering-Chukchi-Beaufort Seas bowhead population. Although analyses of preliminary data identified some potential spatio-temporal substructure, final analyses of a better, larger dataset found no evidence that the population should be treated as being comprised of multiple stocks. A "generational gene shift" hypothesis (Archer et al., 2010) proposed that non-random sampling of a single population strongly out of genetic equilibrium due to the impacts of heavy commercial

whaling less than one bowhead lifetime ago can lead to statistical results that would be misinterpreted as indicating stock substructure. In the second half of his talk, Givens described the 2011 ice-based visual and acoustic bowhead census, and the statistical population abundance estimation methodology. The counts of sighted whales are corrected for three factors: availability (for a whale to be seen), detectability (given that the whale is available), and effort (since the visual perch was sporadically inoperational). The details are given by Givens et al. (2016), who estimate a 2011 abundance of 16,820 whales (95% confidence interval (15,176 to 18,643)) increasing at an annual rate of 3.7% (2.9% to 4.6%).

Manoj Samanta. Answering long-standing evolutionary questions using the latest genomic technologies - our electric fish experience.

Rapid fall in the cost of DNA sequencing has allowed scientists to begin addressing many long-standing evolutionary questions at the molecular level. One such question is how some fish species can generate very high electric field and use it for sensing and attack. In his book, Darwin mentioned it as a vexing challenge to his theory of evolution. We recently took the advantage of low-cost sequencing to assemble the genome of Amazonian electric eel and the transcriptomes of other evolutionary distant electric fish. Based on our electric fish experience, I will discuss various approaches for the bowhead whale community to follow to move beyond genome assembly, and connecting to interesting biological questions.

Andrei Seluanov. Comparative Genetics of long lived and cancer resistant species in biomedical research

In the past decade comparative genetics was successfully used in our lab for identification of several novel molecular mechanisms of longevity and cancer resistance. One of the best examples of such mechanism is accumulation of the high molecular mass hyaluronic acid in the naked mole rat. Identification of the completely different mechanism of cancer resistance in the blind mole rat indicates that different mechanisms evolved independently in different long-lived and cancer resistant species. Bowhead whale is a unique species because of its extreme longevity of 211 years and body mass of 60 tons. In collaboration with Drs. Craig George and Vera Gorbunova our lab initiated characterization of Bowhead whale primary cells for identification of novel longevity and cancer resistance mechanism/s. We currently established a tissue culture condition for Bowhead whale skin and lung primary fibroblasts and performed preliminary characterization of Bowhead whale cell growth.

Hans Thewissen. The Sense of Smell in Whales

We investigated olfaction in bowhead whales by studying the anatomy, microanatomy, and genome. Bowhead whales have an olfactory bulb that is larger than that of most other cetaceans. The proportion of the brain that is made up of the olfactory bulb is similar to that in old world monkeys, and considerably larger than in apes or humans. As in other mammals, the olfactory bulb of bowheads has 8 layers, and appears functional. Approximately 50% of the olfactory receptor genes in bowheads are pseudogenized, similar to that of minke whales, and much less than in studied odontocetes (50-100%). However, in artiodactyls pseudogenization affects less than 20% of olfactory receptor genes. We conclude that bowhead whales have the anatomical

and genomic hardware that makes olfaction possible, and that their sense of smell, although small compared to other mammals, is greater than other cetaceans.

Xuming Zhou. Genomics and transcriptomics provide insights into evolution and adaptations of marine mammals

The genetic adaptation, genetic diversity, and demographics of cetaceans are issues that biologists eager to resolve. By analyses of Yangtze River dolphin genome, tanscriptome of bowhead whale, and representative genomes of marine mammals, this presentation has shown that cetaceans have a slow molecular clock and molecular adaptations to their aquatic lifestyle. We also reconstructed the ancient demographics of Yangtze River dolphin and find a significantly lower number of heterozygous single nucleotide polymorphisms in this functionally extinct dolphin compared to all other mammalian genomes reported thus far. With regard to bowhead whale, based on the *de novo* assembly of its transcriptome, we reported the bowhead whale or cetacean-specific changes in gene expression in the liver, kidney and heart; changes associated with altered insulin signaling and other gene expression patterns could help explain the remarkable longevity of bowhead whales as well as their adaptation to a lipid-rich diet. Finally, we have revealed the convergent phenotypic changes in marine mammals (cetaceans, pinnipeds, sirenians) are mainly driven by independent substitutions in common genes and relaxed negative selection rather than parallel substitutions. These data are valuable resources for the study of this remarkable group of mammals and conclusions give insights to many areas such as molecular evolution, genomics, and medical sciences.

Discussion of the outcomes of the meeting.

The meeting provided a great opportunity for networking and communication among scientists and stakeholders, including through formal presentations, panel discussions, and social events. Additionally, important information was exchanged on current methods of genomics analysis and the rapid pace at which this science is progressing. Valuable insight was obtained by the NSB bowhead research team with regards to the development of advanced methods to conduct population genomics analyses.

Both the research community and the NSB had an opportunity to present their perspectives on the challenges of conducting research on bowhead whales. Each in turn benefitted by this exchange. The NSB and AEWC discussed the need to protect the rights of the community to derive benefits from their natural resources, and the research community discussed the importance of bowhead whales as an animal model for the study of disease and aging from which all of society might benefit. The NSB and the Department of Wildlife Management emphasized the need for researchers to justify their work based upon a clear benefit to the local community and to the goals of the NSB mission statement. And it was concluded that a better system of loaning materials and tracking their use among researchers is needed.

In sum, the meeting was highly successful, collegial and the participants were exposed to an invigorating mix of cutting edge genomics research, fascinating stories of the evolution of whales, bowhead whale biology and management, and the history of the Inupiat/Yupik culture.

ACKNOWLEDGMENTS

The bowhead genomics workshop was funded by the NSB and the AEWC. The Battelle Memorial Institute hosted the meeting at their Columbus, Ohio campus. We thank the many people at both Battelle and the NSB who helped to make this event possible. And, we thank the participants of the meeting for providing outstanding presentations and enlightened discussions about bowheads, genomics and related topics.

APPENDIX 1

Meeting Participants

Amy Baird
University of Houston – Downtown
bairda@uhd.edu

Scott Ballinger
University of Alabama at Birmingham
Birmingham, Alabama 35294
sballing@uab.edu

John Bickham
Battelle
Houston, Texas 77079
bickhamj@battelle.org

Harry Brower
North Slope Borough
Barrow, Alaska 99723
Harry.brower@north-slope.org

Anna Brueniche-Olsen
Purdue University
Lafayette, Indiana 47901
annabo@utas.edu.au

Lisa Noelle Cooper
NEOMED
Rootstown, Ohio 44272
lcooper@neomed.edu

J. Andrew DeWoody
Purdue University
West Lafayette, Indiana 47907
dewoody@purdue.edu

Mike Dickens
Battelle
Columbus, Ohio 43201
dickensm@battelle.org

Craig George
North Slope Borough
Barrow, Alaska 99723
Craig.george@north-slope.org

Geof Givens
Givens Statistical Solutions, LLC
Fort Collins, Colorado 80526
geof@geofgivens.com

Bernhard Metzger
Battelle
Lexington, Massachusetts 02421
metzgerb@battelle.org

Manoj Samanta
Homolog
Redmond, Washington 98053
samanta@homolog.us

Michael Scott
ExxonMobil Development Company
Spring, Texas 77389
Michael.j.scott@exxonmobil.com

Andrei Seluanov
University of Rochester
Rochester, New York 14627
Andrei.seluanov@rochester.edu

Gary Stuart
Indiana State University
Terre Haute, Indiana 47892
gstuart@indstate.edu

Robert Suydam
North Slope Borough
Barrow, Alaska 99723
Robert.suydam@north-slope.org

J.G.M. 'Hans' Thewissen
NE Ohio Medical University
Rootstown, Ohio 44272
thewisse@neomed.edu

Alan Tilstone
Battelle
Burlington, Massachusetts 01803
tilstonea@battelle.org

Xuming Zhou
Harvard Medical School
Boston, Massachusetts 02115
Xmzhou0103@163.com