Report from
Bowhead/Right Whale Comparative Health and Physiology Workshop
March 25-26, 2003

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1. Executive Summary:

Bowhead whales (*Balaena mysticetus*) and right whales (*Eubalaena* spp.) are closely related phylogenetically, and they have many similarities in morphology and behavior. As a consequence, comparative approaches to research should be productive. Researchers studying the highly endangered North Atlantic right whale (*E. glacialis*), a fully protected species, have expressed interest in using the bowhead whale, a species hunted for subsistence by Native people in Alaska, northern Canada, and eastern Russia, as a surrogate or model for addressing certain questions and for technique validation, particularly when access to fresh whale carcasses is required. Similarly, bowhead whale researchers have long been interested in the findings of right whale researchers who study a small, living population in which most individuals have been photo-identified. The need for improved understanding of physiology and individual animal health has been cited as a priority by a number of meeting and workshop reports concerning both bowhead and right whales. In addition, recent evidence of poor body condition along with a two-year spike in observed mortality have caused concern about the health status of eastern Pacific gray whales (*Eschrichtius robustus*), and insights from studies of bowhead and right whales may prove relevant for that species (and vice versa).

A Bowhead/Right Whale Comparative Health and Physiology Workshop was convened in Boston, Massachusetts, in March 2003, with the stated goals of:

- Determining the measurable physical, chemical, biochemical, and physiological parameters that would be used to assess the health of bowhead and right whales, and
- Establishing priorities for comparative investigations of mysticete nutrition, health and biology.

Objectives of the workshop were to:

- Review ongoing studies, identify new areas for collaborative research, and develop specific hypothesis-driven comparative studies of right whales and bowheads to assess health, physiology, nutritional status, and reproductive biology.
- Develop long-term standardization of sampling and data collection to allow comparisons.
- Assess the rationale of the bowhead/right whale comparative model for different research areas.

A previous workshop in Barrow, Alaska, in October 2001 had been intended to cover much of this agenda. However, due to the disruptions caused by the terrorist attacks in Pennsylvania, New York and Washington, D.C., in September 2001, it proved impossible to assemble the full complement of key researchers. Nevertheless, the proceedings of the Barrow workshop (Willetto et al. 2002A) should be viewed as a companion document to the present one. Also, important background to the present workshop can be found in the reports of two previous workshops, one on Status and Trends of Western North Atlantic

Right Whales in 1999 (IWC 2001B) and one on Causes of Reproductive Failure in North Atlantic Right Whales: New Avenues of Research (Reeves et al. 2001A).

The first part of the present workshop consisted of presentations on the status of bowheads in Alaska, and of right whales in the western North Atlantic. This was followed by five sessions that focused on the links between health/reproduction and specific factors: (1) feeding ecology/nutrition, (2) genetics, (3) stress, (4) contaminants/toxicants, and (5) pathology/disease. Abbreviated sessions addressed marine biotoxins and age-estimation methodology.

Consideration was given to the comparative advantages and disadvantages of studying live, free-ranging animals, fresh carcasses of hunter-killed whales, and stranded (and therefore often autolyzed) carcasses to investigate health, condition, and physiology. Among the clearest advantages of studying live whales is that it allows multiple sampling, whether through photo-identification, remote biopsy sampling, or tagging. With such studies, it is possible to assess impacts over time of various stressors on behavioral endpoints such as respiration, feeding, and responses to noise or toxicants (i.e. biotoxins). In a situation where all, or most, individuals are known, it may also be possible to assess the impacts of some stressors on reproduction and mortality. In contrast, studying dead animals has the advantage of allowing greater control over and extent of tissue sampling as well as whole-body examination, inside and out. Disease processes and diagnostics can often be addressed most directly with fresh carcasses or, in the case of strandings, moribund animals just prior to death or euthanasia.

Five working groups were established to develop testable hypotheses, where feasible, and to identify the preferred population, methodology, and current data to be used for hypothesis testing. The *validation/calibration group* produced a series of measures or indices, each matched to one or more approaches for validation (Appendix 1). The *genetics group* offered several hypotheses, one suggesting that low allelic diversity in the Major Histocompatibility Complex (MHC) is limiting the potential reproductive capacity of the North Atlantic right whale population, and another that this same limited allelic diversity is responsible for a higher disease incidence in that population compared with other balaenid populations. Other hypotheses related to bowhead stock and "sub-stock" structure were discussed. The *working group on methodology standardization* outlined ways in which data collection could be standardized across studies of the different species, noting that this subject might require a separate workshop. The basic hypothesis stated by the *working group on toxicants and disease* was that reproductive success is affected by infectious or non-infectious disease. This group also noted that recently developed health assessment procedures will allow for more focused efforts to assess cause (diagnostics) and impacts. Finally, the working group on *environmental factors and nutrition* proposed three hypotheses: (1) that variability and trends in balaenid population parameters and habitat use are linked to both regional and broad-scale climatic and oceanographic patterns; (2) that balaenid reproductive rates and parameters, as well as survivorship, are dependent upon food availability and predictability of food supply; and (3) that human activities (e.g., shipping, fishing, seismic exploration and other noisegenerating activities, dredging, production of coastal effluents) will alter balaenid habitat-use patterns, and secondarily affect reproduction and survivorship.

The workshop concluded that there was enormous potential for synergy from the ongoing and planned research on bowhead whales and right whales and that further collaboration should be encouraged. Specific recommendations were:

1. Efforts to study living bowhead whales in the Chukchi and Beaufort Seas, using biopsy sampling, photo-identification, tagging, and other techniques, should be expanded to obtain baseline data in relation to reproductive and health status.
2. Correspondingly, efforts to obtain fresh biological materials and other data (e.g., standard measurements) from dead right whales should be enhanced.
3. Research programs with bowhead, right, and gray whales should be encouraged to standardize data collection procedures to the maximal possible extent, e.g. by exchanging and adapting data sheets, establishing collaborations in comparative studies, etc.
4. Ongoing long-term studies of photo-identified right whales should incorporate health assessment components, such as recording of external (skin) lesions, fecal and biopsy sampling, and measures of blubber quality and quantity.
5. Efforts to detect and respond to strandings of bowhead (and gray) whales should be strengthened to improve the chances of establishing cause(s) of deaths.
6. For bowhead (and gray) whale populations, catalogues of high-quality photographs of live animals should be maintained more rigorously to support studies of scarring, reproductive success, etc.
7. Oceanographic (chemical, physical, and biological) studies are proposed to address variations in whale behavior, distribution, reproduction, survivorship, and health status over time and space (i.e., “whale-centric” oceanography).

2. Introduction

On March 25-26, 2003, a group of scientists and managers assembled to discuss "comparative health and physiology" of the bowhead whale (*Balaena mysticetus*) and North Atlantic right whale (*Eubalaena glacialis*). The meeting focused on the Bering-Chukchi-Beaufort Seas (BCB) stock of bowhead whales and the western population of North Atlantic right whales (NARW) (Agenda as Appendix 2). This report on the 2003 workshop in Boston builds upon the report of a previous workshop in Barrow, Alaska (October 2001) that focused on the bowhead whale (See Willetto et al. 2002A and 2002B). Due to the unfortunate circumstances surrounding September 11, 2001, participants from the New England area and outside the United States were unable to attend the Barrow Workshop, and it was intended that the Boston workshop would address this deficiency. Four residents of Barrow (scientists and hunters) participated in the Boston workshop (See Appendix 3 for list of participants).

The report of the International Whaling Commission's (IWC) 1998 Workshop on the Comprehensive Assessment of Right Whales (IWC, 2001A) contains a series of recommendations regarding factors potentially affecting population recovery. These factors include inbreeding depression, trophic structure and productivity, body condition, chemical pollution, mortality from entanglement in fishing gear and ship strikes, habitat loss, climate change, and disease. Another followed the 1998 Workshop in October 1999 on Status and Trends in North Atlantic Right Whales (IWC 2001B), and by another in 2000 on Causes of Reproductive Failure in North Atlantic Right Whales (Reeves et al., 2001A). IWC (2001B) provides information suggesting significant declines in abundance and reproduction in the NARW population over the last 20 years. Recommendations of that workshop included the need to eliminate anthropogenic mortality, and to expand research on the causes of reproductive decline (see Table 5, p 75, IWC 2001B). Similar but more detailed recommendations were made by Reeves et al. (2001A), and several relevant studies have been initiated since 2000 when those recommendations were formulated. A summary of the factors considered and the current status of related studies is given in Table 1, and basic life history information on bowhead whales and right whales is presented in Table 2.

It was generally agreed that valuable insights would be gained by combining and comparing the research efforts that are underway and planned for bowhead whales and right whales (e.g. Table 3, later).

Workshop Goals and Objectives

The *goal* of the Boston workshop was to determine the measurable physical, chemical, biochemical, and physiological parameters that would be used to assess the health of bowhead whales and right whales, and to establish priorities for comparative investigations of mysticete nutrition, health and biology.

The *objectives* of the workshop were to:

1. Review ongoing studies, identify new areas for collaborative research, and develop specific hypothesis-driven comparative studies of right whales and bowheads to assess health, physiology, and nutritional status.

2. Develop long-term standardization of sampling and data collection to allow comparisons.

3. Assess the rationale of the bowhead/right whale comparative model for different research areas.

3. Presentations Reviewing Ongoing Studies (follows order of workshop agenda)

3.1 Western Arctic Bowheads: Barrow Workshop Summary: Willetto

The 2001 workshop in Barrow and the associated published report was reviewed (Willetto et al., 2002A. and 2002B). The workshop report has been circulated to bowhead whale hunters, local and federal agencies, and sponsors; pdf files have been produced that are available on CD; and all of this material was presented to the IWC Scientific Committee (Willetto et al. 2002B), to the Eskimo Walrus Commission (EWC), and to participants at the Boston workshop prior to the meeting. The underlying reasons for examining landed bowhead whales are to: evaluate population status and health; assess the health of individual animals; improve hunting efficiency, human nutrition, and food safety; and obtain data on basic biology. This presentation emphasized that the key to success is the cooperation of the hunters and scientists. It is critical to understand the extent of “natural variability” in the many condition and health indices that are evaluated and to realize that some variation is normal and to be expected. This natural variability occurs both among individuals and within individual animals, and it greatly influences interpretations of health, condition, and other data.

A better understanding of natural variability is improving how we interpret data, including those associated with health and condition such as measures of blubber (a keystone matrix) – e.g. depth, lipid content, thermal conductivity, stratification of blubber components, etc. Blubber assessment has demonstrated the need to better understand variability using standardized methodology. This presentation provided necessary background to ensure that the workshop would follow up on the efforts made in Barrow in 2001. For further details, readers are referred to Willetto et al. (2002A and 2002B).

3.2 Status of Eastern Arctic Bowheads: Research and Management Summary: Cosens

Historical patterns of whaling effort and modern genetic evidence suggest that there are two stocks of bowhead whales in the eastern Canadian Arctic, the Hudson Bay-Foxe Basin (HB-FB) stock and the Baffin Bay-Davis Strait (BB-DS) stock. Over-harvesting by commercial whalers through the end of the 19th century depleted both stocks, and they are currently listed as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC).

The HB-FB stock uses two main summering areas – waters around Southampton Island in northwestern Hudson Bay and northern Foxe Basin. Satellite-tracking data show that at least some whales move from Foxe Basin through Fury and Hecla Strait into the Gulf of Boothia. Whales from this stock are thought to over-winter in the vicinity of Hudson Strait, but their exact wintering locations are not known.

The BB-DS stock occupies high arctic waters in Canada during the summer. Although these whales are known to over-winter off the west coast of Greenland, surveys in March have found bowheads in the vicinity of Cumberland Sound and at the east end of Hudson Strait. It is possible that these whales belong to the BB-DS stock.

The Inuit Bowhead Knowledge Study concluded that people were seeing more bowheads in the 1990s than they had seen 20 or 30 years earlier. Scientific data collected since 1994 are beginning to corroborate the view that bowhead stocks are recovering from commercial whaling.

3.3 North Atlantic Right Whales (NARW): Research Summary: Kraus

The right whale population in the western North Atlantic currently numbers about 300 animals (IWC 2001B; Kraus et al. 2001) and appears to be declining (Caswell et al. 1999; Fujiwara and Caswell 2001). Many western NARW have died from to anthropogenic causes, and the population has experienced a significant decline in reproductive rate or a significant adverse disease or food limitation event during the past decade (Knowlton and Kraus 2001; Kraus et al. 2001).

There are five well-known right whale habitats along the east coast of North America. Most cows give birth in the coastal waters of the southeastern U.S. during the winter (Kraus et al. 1986). Males and non-calving females are rarely seen in that area, and their winter distribution remains unknown (Kraus et al. 1988). In the spring, aggregations of NARW are observed in the Great South Channel east of Cape Cod, and in Massachusetts Bay (Winn et al. 1986; Hamilton and Mayo 1990; Kenney et al. 1995). In the summer and autumn, they are observed in the Bay of Fundy between Maine and Nova Scotia, and on the Scotian Shelf 50 km south of Nova Scotia (Stone et al. 1988; Kraus and Brown 1992). Several sightings of NARW have been reported from the Gulf of Mexico to Greenland (Knowlton et al. 1992), and an additional summering habitat has been proposed based on sightings and genetics data (Malik et al, 1999).

The NARW population was probably smaller in the recent past. Malik et al. (1999) found only five matrilines represented in the mitochondrial DNA (mtDNA) from over 200 animals sampled in the western North Atlantic. Since mtDNA is maternally inherited, this suggests that the population went through a very small "bottleneck". The catch history suggests that the population reached very low levels by the early 1900s (Reeves et al. 1992, Reeves et al. 2001B).

Documented right whale deaths between 1970 and 2002 indicate that 6/55 (10.9%) were due to entanglements in fishing gear, 18/55 (32.7%) were due to collisions with ships, and 16 (29.1%) and 15 (27.3%) were attributed to "unknown causes" or "neonatal mortality", respectively. Over 70% of all right whales have scars indicative of entanglements.

Causes for the reproductive decline are uncertain, and studies are currently underway to examine the potential effects of contaminants, biotoxins, disease, prey limitations, and genetics (see Reeves et al., 2001A). Pollutants are present in measurable amounts in right whales (e.g., Weisbrod et. al. 2000), but direct causal links between various compounds and health, reproduction, or behavior have not been identified.

3.4. Feeding Ecology, Nutrition, Blubber Studies: Relationship to Health and Reproduction

3.4.1. Bowhead Current and Future Research: George/O'Hara

A bowhead whale feeding study in 1985 and 1986 in the Eastern Alaskan Beaufort Sea (EABS) concluded that bowhead whales derive only a very small portion (~1%) of their annual nutrition from this area. A second feeding study in the EABS (1998-2000) used the same basic methodology (boat-based plankton tows, analysis of whale stomach samples, aerial surveys, and whale photo-identification) with some major modifications including a scientific review board (SRB), a local knowledge component; more advanced statistical and whale energetics analysis, analysis of stomach samples from Barrow for comparison to Kaktovik landed whales; and expanded chemical analysis techniques (carbon isotopes and fatty acid profiles) of whale tissue for interpretation of feeding history (Richardson et al., 2002 "Feeding Study").

The basic findings are that: (1) whales feed 47% of the time while in the EABS; (2) densities of zooplankton over much of the area are sufficient to support whale feeding (>800 mg/m^3); (3) 83% of the whales landed at Kaktovik had been feeding; (4) based upon isotope analysis of the muscle, sub-adult bowheads derive 14% (95% CI 4% to 23%) of their total nutrition from the entire Central and Eastern Beaufort Sea; and (5) feeding in the study area "varies widely between years and areas within the EABS.

The use of stable isotopes in muscle and OCs in blubber was reviewed to assess feeding ecology as it relates to animals examined in the fall versus spring hunt, and between locations in the fall (Kaktovik and Barrow) (see Hoekstra et al. 2002C). These data clearly show active feeding of bowhead whales in the Beaufort Sea along the northern coast of Alaska. Organochlorine concentrations are relatively low. Levels in whales are higher than in prey and water (indicating bioaccumulation), biomagnification is greater in males than females, and levels vary by season due to variations in local contamination of the Bering Sea versus the Beaufort Sea (Hoekstra et al. 2002a,b). These studies demonstrate the utility of applying basic chemical tools to whale research programs and offer insight to whale feeding ecology.

Intensive evaluation of blubber in the bowhead whale (i.e., assessment of 30 samples per whale for approximately 50 whales) has indicated significant natural variability in the population (e.g., decreasing % lipid content in male bowhead whales with increasing body length, stratification of % lipid content by depth of blubber sampled). Research on bowhead whale blubber by Ylitalo (lipid and lipid classes), Mau (proximate composition), Rosa (histology), and George (physical properties) is contributing to a better appreciation of bowhead whale blubber composition and function by addressing this "natural variability." See Willetto et al. (2002A and 2002B) for a review of those efforts. The detailed blubber research in bowhead whales should facilitate studies of blubber in other large mysticetes. Nutritional assessment must include matrices other than blubber and incorporate essential elements in many tissues. It must also include muscle mass (girth measures), histology (fiber size and type), biochemistry, and digestive organ histology. The nutritive value (lipids, vitamins, minerals, etc.) to consumers is a high priority to Native Alaskans. Publications, current data, and ongoing work were reviewed.

3.4.2. Right Whale Feeding Ecology Current and Future Efforts

3.4.2.1. Blubber Thickness Studies and Reproduction in Right Whales: Moore

To investigate the role of nutrition in reproductive performance, blubber thickness was measured acoustically in free-ranging right whales (Moore et al. 2001). Data indicate that the more reproductively successful South African right whale group has significantly thicker blubber than the NARW. In South African right whales, lactation and suckling affect blubber thickness, suggesting that blubber supports reproduction and nurturing and mirrors energy balance in right whales. In NARW, recently lactating mothers have low reserves (thinner blubber) compared to non-parous females. Non-parous have thinner blubber than overdue mothers, suggesting that non-calving females in the NW Atlantic may have insufficient blubber reserves to support reproduction. Blubber thickness increased over 1998, 1999, and 2000 in adult NARW, an observation that may help explain the successful calving season in 2001 (31 calves).

Currently we are standardizing our acoustic data extraction protocol, normalizing thickness measurements across the body, and completing analysis of our database. Development of a right whale body condition index is underway in collaboration with Perryman and Pettis, integrating blubber thickness and composition, body length and girth, and visual assessment. Future plans include expansion of field studies to other NW Atlantic habitats, such as the Great South Channel, and potentially, southern right whale feeding habitat off South Georgia (Moore et al. 1999). In terms of building a collaboration between right whale and bowhead research resources, it was suggested that we should assess: (1) whether the ultrasound can assist with the estimation of bowhead hypodermal thickness, and (2) the feasibility of sampling lipid content from the acoustic signal echoes in the blubber of recently dead bowhead carcasses to allow calibration of ultrasound echo pattern to lipid content. Studies of body condition in live bowheads to allow calibration of acoustic and photogrammetric body condition indices should be pursued.

3.4.2.2. Linking Food Resources to Reproduction for Right Whales: Mayo

The relationship between calving and food availability for right whales was discussed. The tested hypothesis (#1) was: Calving success of right whales in the western North Atlantic reflects the quality of the Cape Cod Bay food resource.

To test hypothesis #1 we determined the quality of the zooplankton resource by examining data from 1986 – 2000 and calculating the Caloric Capture factor (CCf). Direct comparison of the CCf to calving rate by year using the Spearman Rank Correlation Coefficient (SRCC) of trends demonstrates that there is no correlation [SRCC = –0.006 (p>0.1)]. Lagging the data by 2 years gives the whales time to "respond" to previous nutritional conditions. The SRCC for a comparison of all NARW calving data lagged by 2 years versus the CCf is 0.739 (p = 0.00083).

Extension of the original hypothesis results in testing hypothesis #2: The association between calving success and the food resources of the Bay should be seen most strongly in the calving by mothers who depend on the resources of the Gulf of Maine and not by those mothers who depend for nutrition on areas inferred to be far removed from the Gulf and the Bay.

Comparing 2-year lagged CCf to calving data for the non-Bay of Fundy mothers demonstrates a lack of association with the CCf. However, the comparison of the calving mothers seen both in Cape Cod Bay and the Bay of Fundy vs. the CCf has a SRCC of 0.803 (p<0.001), suggesting a high degree of association between the patterns of the CCf and calving by the Gulf of Maine/Bay of Fundy mothers.

The conclusion is that over the last 15 years there has been a strong relationship between the measures of caloric availability in Cape Cod Bay and the calving of the NARW lagged by 2 years. Further, the correlation is very strong for mothers with an affinity for the Gulf of Maine but not significant for those believed to obtain most of their nutrition elsewhere.

3.4.2.3. Linking Climate (NAO) to Right Whale Reproduction: Kenney

Population growth rate for the NARW, even during the "good years" in the 1980's, is substantially lower than rates observed in southern right whales. Interannual variability in NARW reproductive success is very high, with the minimum and maximum numbers of calves, 1 and 31, occurring in 2000 and 2001, respectively. From 1982 to 1992, the average number of documented calves per year was 12.4 (CV=24.1%). Through 2001, the average was essentially unchanged at 12.3, but the CV more than doubled to 55.4%. Prey availability is likely to vary significantly in the short term, possibly influencing reproduction by affecting the ability of mature females to accumulate the fat reserves necessary for supporting a fetus and a neonate.

The time series of calves per year is visually similar to the time series of the Southern Oscillation Index (SOI), a measure of South Pacific atmospheric pressure patterns linked to the El Niño phenomenon. There is a statistically significant correlation between NARW calving and the SOI lagged by one year ($R = 0.484$, $p = 0.031$). Low calving years follow one year after El Niño years. There is no significant correlation between NARW calving and the North Atlantic Oscillation (NAO), a similar atmospheric pressure phenomenon. Partitioning the calves into "Fundy" and "non-Fundy" cohorts, however, results in a very weak correlation between calving and the 1-year-lagged SOI for the Fundy cohort ($R = 0.404$, $p = 0.077$), and a significant correlation between non-Fundy calving and the winter NAO lagged by 2 years ($R = 0.479$, $p = 0.038$).

The mechanisms of the NAO effects on *Calanus* and therefore on right whale foraging success are not clear, and likely to be complex. Temperature affects the growth, metabolic rate, feeding activity, food availability, and reproduction of *Calanus*. Current patterns influence the "supply" of *Calanus* to the system from slope waters, as well as the physical mechanism(s) responsible for aggregating them into the dense patches that right whales require for successful feeding. A number of complexities will need to be better understood and incorporated before we can take the next step from simple correlations to a more useful, quantitative, predictive approach. It is likely that the observed effects are a complex of opposing positive and negative effects of temperature and currents.

4. Genetics

4.1. Review of Right Whale Genetic Studies: White

There are three primary questions:

1) Is the poor reproductive performance (an average of 11.7 calves/year compared with an expected 33) of the NARW due to low haplotypic variation at the Major Histocompatibility Complex (MHC)?
2) Are there two sub-populations that are reproductively isolated?
3) Where are the mating grounds and second nursery?

DNA samples from more than 270 known animals and 50 more from other animals (some yet to be photo-identified) have been collected. These are being profiled at 37 microsatellite loci, the mitochondrial control region, Zfx/y, and the MHC loci. There is very low genetic variability in this species, and a large number of loci are required to assess paternity. High probability paternity assessment is required to test the hypothesis that there is an elevated level of spontaneous abortion due to the fetus's having a high probability of genetic similarity to the mother at the MHC. We have profiles on all of the animals at 21 microsatellite loci, and the preliminary paternity assignments indicate a large proportion of the males are gaining fertilizations. These data will be used to determine the effective population size. The data also suggest a level of reproductive isolation between the group using the Bay of Fundy as a nursery and the group that uses an alternate nursery. A major problem is the low percentage of samples from the group that does not use the Bay of Fundy as a nursery. We will be concentrating on increased sampling in the southeast where the calves are born as well as the Great South Channel and Cape Cod Bay. In the early 1990s, the use of Roseway Basin off Nova Scotia declined, and this coincided with increased use of the Bay of Fundy and a subsequent decline in the reproductive performance of the Bay of Fundy group. The existence of two groups allows comparative analyses to assess effects of habitat use on reproduction and health. Integration of the genetic profiles with the photo-identification catalogue is underway in order to predict possible locations of mating grounds as well as the other nursery area. The DNA profiles are also being used to refine the census size of the population especially by assessing new animals that are added to the catalogue. This is being done by assessing maternity of these animals and known calving events where the mother-calf link was not made by photo-identification. We are also assessing the historic levels of genetic variation in both right whales and Eastern Arctic bowheads using DNA isolated from bones of whales harvested by Basque whalers in the 16th century.

4.2. Review of Bowhead Genetic Studies: O'Hara/George

A brief review of genetic work conducted on the BCBS stock was presented. Rooney et al. (2001) inferred historical change in population size from DNA sequence polymorphism data. Rooney (1998) and Rooney et al. (1999) evaluated the putative bottleneck in the BCBS population using patterns of microsatellite diversity and genetic disequilibria. They concluded that there was no evidence of a bottleneck, and concluded that bowheads may have increased in abundance with reduced arctic sea ice coverage.

Other basic scientific questions have also been addressed: Rooney et al. (2002) conducted a phylogenetic and morphometric analyses of the *ingutuk* (very rotund bowhead whale) and concluded that it most likely represents a post-weaning phase of normal bowheads and is not genetically distinct from non- *ingutuks*. Other studies comparing bowhead whale stocks have been conducted and are underway.

The next critical question facing BCBS bowhead whale management that may be addressed using "genetic tools" is that of BCBS substock structure. Specifically, are the whales in Russia in spring a genetically distinct group from those traveling along the northern coast of Alaska in spring? This stock question could affect strike allocations in the Bering Sea region. Hunters are collecting samples and helping investigators begin to address it.

5. Reproduction and Stress
Calf production indices for assessing reproductive "health"

5.1. Right whales: Kraus

Most NARW calves are born in southeastern U.S. coastal waters (Kraus et al. 1986). Gestation is estimated to last 12-13 months based upon studies of southern right whales (*Eubalaena australis;* Best 1994). In 1992, the mean interval between births was 3.67 years (n=86) (Knowlton et al. 1994), with a range from 2 years to 7 years. However, in the 1990s the mean calving interval increased significantly to nearly 6 years (Kraus et al. 2001). This increase was associated with increased variability in annual calf production.

At least two females have had calves over a period of 28 years. Since 1990, the number of calves produced each year has varied from 1 to 31, with no apparent trend (Figure 1).

Malik et al. (1999) and Schaeff et al. (1993) inferred from genetic and photo-identification data that one group of cows does not bring calves to the Bay of Fundy each year. Therefore, at least one other summer and fall nursery area must exist. No such nursery is in the high-use area of the Scotian Shelf near Browns Bank, because cow and calf pairs were seen there only four times in 1,059 sightings over eight field seasons (Knowlton et al. 1995). Knowlton et al. (1992) reported a cow and calf south of Greenland in 1992, and historical whaling records suggest an area between Greenland and Iceland (Reeves and Mitchell 1986) as a candidate nursery.

NARW reproduction has been declining by several measures since about 1990 (Kraus et al. 2001) including calves per mature female per year, intervals between calves, and averaged calf counts/estimated population size. The potential reasons include food limitation (Moore et al. 2001), population and habitat use changes (Kenney et al. 2001), disease and/or marine biotoxins (Rolland, pers. comm.), pollutants (Colborn and Smolen 1996; Weisbrod et al. 2000), genetic factors (Schaeff et al. 1997), and climatic variation (Kenney, this report). However, no direct link between any of these potential factors and the reproductive decline of right whales has yet been established. Individual photo-identification and yearly calf counts are critical to monitoring reproductive performance and are the foundation from which other studies can seek to identify major causes of poor calf production and neonatal losses.

5.2. Bowhead Whales: George/O'Hara

The BCBS bowhead whale population appears to be increasing steadily at about 3% per year based on census data over the past 23 years (1978-2001). However, the reproductive rate of bowheads is low compared with other balaenids yet the rate of increase (ROI) is substantial. This suggests very low natural mortality rates.

Little is known about calving intervals, with only 4 photo-identification "recaptures" of mother-calf pairs. The intervals were 4, 4, 7, and 7 years. The 7-year intervals could actually be combinations of 3- and 4- year birthing cycles. Other data, including crudely estimated pregnancy rates from landed bowhead whales (0.31) and the observed rate of increase (about 3% per year), suggest that cycles average 3 to 4 years.

Based on postmortem examinations of landed whales, an increase in pregnancy rates in recent years is suspected. These data have low statistical power but nonetheless indicate a significant increase (using weighted regression). This could be an effect of reduced ice cover in the Beaufort Sea feeding grounds. There is evidence that bowhead whales are 'fatter' (better body condition) in years with reduced sea ice, and this may be linked with increased observations of calves. Pregnant females tend to arrive later in the migration than non-reproductive females. This may be due to slower swim rates of neonatal calves, or pregnant females, or other factors (Figure 2). Currently, the North Slope Borough is funding aerial photogrammetry work (2003) at Barrow to further address these and other issues, and is continuing annual examinations of landed whales in Barrow and Kaktovik.

5.3. Reproductive and Stress Hormone Studies in Right Whales and Bowheads: Rolland

Studying reproduction in right whales has been problematic because no techniques are available to collect blood or many other samples from large, free-ranging whales. Most stranded animals are very decomposed and tissues are useless for reproductive status analysis. We have validated radioimmunoassay techniques to measure the metabolites of reproductive and adrenal steroid hormones ("stress" hormones) in right whale fecal samples. Using this method, we can measure metabolites of estrogen, progesterone, testosterone and glucocorticoid hormones. The amount of hormone metabolite in feces reflects the average level of the parent hormone in the blood during the previous day or earlier, depending on hormone clearance rates and gastrointestinal transit time.

From 1999-2001, 62 fecal samples were collected from right whales in the Bay of Fundy. Over 60% of these samples were linked to an identifiable whale, allowing correlation of hormone results with age, sex and reproductive history using data from the NARW Catalogue (Hamilton and Martin, 1999). Our results show that measurement of fecal reproductive hormones can be used to distinguish between sexes and reproductive states, provide information on pregnancy, lactation, sexual maturation of males and females, and potentially estrous cyclicity and seasonality of reproduction. Comparison of hormone levels to the reproductive history of individual whales is being used to investigate the physiological basis of the reproductive dysfunction in this population, including the possibility of an abnormally high rate of abortions or stillbirths, reproductive senescence, delayed puberty and infertility. The fecal glucocorticoid assay is still under development, but initial results show that stress hormone levels vary with sex and reproductive status. A sample from an entangled right whale in poor condition showed a three to four fold elevation of glucocorticoids, demonstrating the usefulness of this assay for detecting high levels of physiological stress. Measurement of stress hormones has great potential as a biomarker of health status and as a quantifiable measure with which to explore the impact of environmental stressors on right whale and bowhead whale health and reproduction.

Validation of assays for the same four hormones in blood, feces, and other samples collected from the western Arctic bowhead whale during the spring and fall hunts in Alaska has been completed. Bowheads are being used as a reference population for the right whale studies because (1) the two species are closely related, (2) the bowhead population is growing and reproductively healthy, and (3) both blood and fecal samples can be collected. Hormone levels will be compared with the reproductive condition of the whale through direct examination of the reproductive tracts, and some histologic characterizations (e.g., seminiferous tubule diameters). Since 2000 we have collected fecal and serum samples from 36 bowhead whales of known sex and reproductive condition. Preliminary results indicate that fecal reproductive hormones can be used to determine sexual maturity and /or seasonality of reproduction in males and females and to detect pregnancy in early gestation. Fecal glucocorticoids appear to vary with sex and reproductive condition, supporting findings in right whales. This comparative study will provide baseline data with which to assess the relationship between fecal hormone levels and reproductive condition in large baleen whales that can serve as a model for studies of reproductive dysfunction in the NARW.

6. Pathology/Disease and Health Assessments

6.1. Visual Health Assessment in Right Whales: Pettis

Assessing the health of free-ranging cetaceans is inherently difficult. Although landed, stranded, and captive animals have provided a variety of information on cetacean body condition and individual health for selected species, few have been useful for evaluating trends in body condition over time. We have developed a non-invasive method of assessing right whale health based on photographs of five selected body parameters: 1) body condition, 2) skin (epidermal) appearance, 3) cyamid type and abundance near the blowholes, and 4-5) rake marks forward of the blowholes (left and right sides scored independently). Photographs for nearly 23,000 sightings of 410 individual whales from 1935-2000 were organized by whale, region, and year and were then examined and scored for each body parameter. Scoring was done on a numerical scale, with higher scores assigned to poorer conditions. A double-blind study was conducted to test the consistency of parameter scoring among researchers. This study showed a moderate to high level of consistency for all scored parameters, indicating that the scoring criteria are sufficiently objective. To test the validity of the body condition scoring, photographs of calving females were inspected for changes in body condition between calving and non-calving years. Female right whales were coded as significantly thinner in years with a calf when compared to years without a calf. Cows were also in significantly better condition in the year before calving than the year after calving, suggesting that the visual health assessment technique is sensitive enough to detect the predicted dynamics of cow body condition. Scoring of the five body parameters of whales presumed to be dead (i.e., not seen for 6 years) were compared to those presumed to be alive to determine if there are distinguishable differences between the two categories of animals. Comparisons were done in 5-year subsets from 1981-1995 to ensure adequate sample size. Both cumulative and individual parameter scores were compared. The cumulative health scores were significantly elevated (indicating poorer health and condition) for presumed dead animals compared to living animals over the entire study period. Body condition proved to be the most consistently significantly different parameter across all years, which further suggests that body condition may be a key visual indicator of health status in right whales. This visual health assessment method provides a new tool with which to monitor health trends in right whales at both the individual and population level. It also appears to have potential prognostic value in determining the potential survival (resighting probability) of an individual. Cohort-specific evaluation could be used to describe overall population health.

6.2. Scarring and Injury Analysis of Right Whales: Knowlton

Three different databases were used to assess the level of human interaction with right whales: the "scarring database," "serious injury database," and "mortality database."

The scarring database is a comprehensive review of all photographed sightings of each individual right whale. Photographs of an individual taken within a season/year are reviewed and 21 different body parts (5 on the head, 2 on the back, and 14 on the tail) are coded for the presence/absence of scars, including those from entanglements or ship strike. For entanglements only, the scarring data were reviewed to determine: total number of entanglement events, number of entanglement events versus known population size, annual rate of entanglement, time frame of entanglement detection, age at entanglement detection, and serious entanglements versus observed population.

Of 413 individual right whales reviewed, 297 (71.9%) have been entangled at least once. A total of 543 separate entanglement interactions have been documented between 1980 and 2000. The number of entanglements per individual ranged from one to as many as five. The rate of entanglement by sex was nearly equal. Juveniles are more prone to getting entangled than adults.

The percentage of presumed living individuals entangled within 3-year periods beginning with 1980-1982 and ending with 1998-2000 ranged from 19% to 39%. In the two most recent 3-year periods, 1995-1997 and 1998-2000, the rate has exceeded 30% suggesting an increasing trend. An annual rate of entanglement, determined by comparing the number of adequately photographed animals seen in both years of a given 2-year period divided by the number that became entangled by the second year, ranged from 12% to 33%.

The criteria for designating an animal as seriously injured from entanglement are: 1) the animal is seen carrying line, 2) the animal has an open wound with a depth estimated to be greater than 8 cm caused by an entanglement, or 3) the entangled animal, or animal with entanglement wounds, appears to be in poor health. The serious injuries are further broken down into fatal, possibly fatal, or non fatal. There have been 50 animals seriously injured from entanglement: 6 fatal, 15 possibly fatal, and 29 non fatal.

The criteria for designating an animal as seriously injured from ship strike are: 1) the animal has propeller cut(s) or gashes which are more than approximately 8 cm deep, 2) there is bone breakage which was determined to have happened ante mortem, 3) there is evidence of hematoma or hemorrhaging, or 4) the ship struck animal appears in poor health. There have been 27 serious injuries from ship strikes: 18 fatal, 2 possibly fatal, and 7 non fatal.

Of the 55 documented right whale deaths from 1970-2002, 18 have been from vessel strikes, 6 from entanglements, and 16 of unknown cause, and 15 of the deaths were of newborn calves (i.e., neonatal complications). Twenty-nine of the necropsy reports included observations beyond morphometrics – 17 adults/juveniles and 12 calves. In 14 of the 17 adult/juvenile necropsies, trauma was a significant finding (10 vessel collisions, 4 entanglements). Evidence of trauma was present in 4 of the 12 stranded calves (all vessel collisions). Four additional ship strike mortalities were determined based on the presence of propeller scars on the dorsal aspect of the body, and two additional entanglement mortalities were determined from other evidence (one whale was hauled up in the entangling gear, and one had a satellite tag attached to trailing gear that stopped transmitting when the animal sank).

In summary, right whales face relatively high levels of human interaction that appear to have an impact on health. These serious interactions appear to be increasing for both entanglement and ship strike. The reasons for this could be related to technological advances including faster, bigger ships, and stronger lines in fishing gear. Policy efforts are being made to protect right whales in relation to both the shipping and fishing sectors.

6.3. Bowhead Skin (Epidermal) Studies: O'Hara

Bowhead whale "skin" (epidermis and blubber) studies have historically recorded scarring patterns and related these to causes of trauma. Some of the microscopic and gross structure of skin has been described (e.g. Henk and Mullan, 1996). Recent and ongoing work is aimed at further characterization of the skin structure as well as improved understanding of the "healing" process, and the infectious causes of lesions and scars. This is a major component of a current Ph.D. project (C. Rosa). Dr. Cheryl Rosa presented this work in detail at the New England Aquarium on March 31, 2003 just following the workshop.

The presentation at the workshop summarized many efforts (Rosa, Mau, Ylitalo) as to what is being assessed in whale epidermis and blubber and this is reviewed in Willetto et al. (2002A, B). Sixty-eight whales landed at Barrow and Kaktovik have been sampled for full thickness blubber cores (outer epidermis to muscle, 1 to 6 sites per whale); the maximum of six cores (complete set) has been taken from 40 of the 68 whales. A substantial set of tissues for histological and chemical analyses was collected from 60 of the whales. These landed whales were sampled at Barrow and Kaktovik. Three major variables need to be addressed (1) Sex/reproductive stage, (2) Age, and (3) Season when harvested. The blubber sampling regimen required samples from six sites on the whale (BD, BL, BV, UD, UL, UV where B = 1 meter caudal to the blowhole or axillary girth, U = umbilical girth, D = dorsal, L = lateral, and V = ventral). Each site was then subsampled at five different depths (20% of full thickness each + epidermis) for analyses to address stratification of components. This study design will allow us to describe the natural variability within the animals individually and within the population of landed whales, and it will establish an important baseline for health and condition measurements.

Epidermal lesions were described. Some of them obviously result from scarring due to trauma. Trauma includes: predators, net and rope wounds, collisions/propeller wounds, abrasion, and others. Other "abnormalities" not directly attributable to physical trauma may indicate disease or variations on "normal" (e.g., "normal" sloughing). Classification by cause could include infection (viral, bacterial, fungal, parasitic), nutritional deficiencies, and neoplastic change. Henk and Mullan (1996) morphologically described epidermal lesions including circular depression, sloughing type, raised roughened areas, roughened flat areas, and depressed irregularly shaped areas.

Preliminary findings indicate obvious impacts on skin due to trauma and infectious agents. Concerns of future changes related to environmental conditions (nutrition, warmer water, shipping and fishing, etc.) could be reflected in the status of “skin” (epidermis and blubber). Many of the above criteria need baseline data (background levels of prevalence) and some understanding of the epidermal and dermal “healing” process and basic structure. Much of this affects the NARW – and “skin” health studies are an obvious area for collaboration between bowhead whale and NARW researchers.

6.4. Epidermal Lesions on North Atlantic Right Whales: Marx

Several types of epidermal (outer skin) lesions have been observed on NARW. Sampling these lesions for histological analyses is very difficult, thus a description of gross physical features was conducted. There are two broad categories—White Lesions and Blister-like Lesions. The White Lesions are all light in color, have indistinct boundaries, and appear to be plaque-like. We describe four different types of White Lesions: 1. Circular--round, vividly white, dense-looking lesions that are found anywhere on the body but most often on the head and just behind the blowholes; they are the most common type of epidermal lesion observed; 2. Outline--grayish, translucent lesions that border a callosity, the lips, or the blowholes; 3. Variable—grayish, translucent, of varying shape and size, and found anywhere on the body; 4. Swath—large (> 45 cm^2) gray to white lesion, most often seen on rostrum and behind blowholes. Because many of the whales with swath lesions are never re-sighted, these lesions may be an indication of severely compromised health.

Blister-like lesions describe both the vesicle and the craterous eruption. Small blister-like are multi-focal and usually clustered; and large blister-like are greater than 10-15cm in diameter, localized (thus far seen only on the back) and appear singly. There are a few other epidermal lesions that do not fit the criteria for the above categories and of those the most noteworthy is the Ulcerative with proliferation—a lesion with raised edges and a papillomatous core.

To assess the occurrence of the White and Blister-like lesions we used data from an analysis of scarring in the entire NARW photographic catalog between 1981 and 2000, in which all scars and marks were coded. The occurrence of white lesions, corrected for effort, significantly increased in the latter half of the 1990’s. In fact, of the 395 records of whales with epidermal lesions, 79% occurred after 1995. There were two peaks in occurrence one in 1997, another in 1999, and mostly different whales were affected in those two years. Significantly more males have white lesions (56.5% males, 36.9% females). For reproductive females with white lesions, those that are pregnant have significantly more than those that are nursing or resting. The Blister-like lesions could not be corrected for effort, but significantly more females were observed with this type of lesion (3.6% female, 1.6% male). For all years combined there was no significant difference in age class, however in 1999 there was a significant increase in white lesions on juveniles (44.7% juveniles, 19.6% adults).

The increase in the occurrence of the white lesions in the latter 1990's could be an indication of either infectious or non-infectious disease. A more detailed analysis of the photos of the epidermal lesions in order to understand progression, recurrence and persistence is planned. We hope to collect samples of lesions for histological, genetic, virology, and cell culture analyses.

6.5. Marine Biotoxins- PSP toxin (Paralytic Shellfish Poisoning) Studies in Right Whales (proposed bowhead work): Rolland

Among the factors identified as potentially contributing to reproductive dysfunction is exposure to marine algal toxins (Reeves et al. 2001). A recent publication (Durbin et al. 2002) demonstrated convincingly that PSP toxins occur in *Calanus finmarchicus* collected in proximity to feeding right whales in the Bay of Fundy, Canada. Fecal material from at least ten different whales (17 samples total) obtained during Aug./Sept. 2001 from right whales feeding in the Bay of Fundy, indicated the presence of PSP toxins by both receptor binding assay and by HPLC-FD analysis, with levels frequently exceeding 0.5 µg STX equiv. per gram of feces. Zooplankton samples (primarily *Calanus finmarchicus*) collected during the same time period also contained PSP toxins. These findings provide compelling evidence for the occurrence of PSP toxins in both right whales and their zooplankton prey, suggesting that additional studies are warranted to examine the trophic transfer of these biotoxins and their possible effects on the reproductive success and on other functions of right whales. A comparative study is underway to test fecal samples from the BCBS stock of bowhead whales for PSP toxins.

7. Age Estimating Studies In Bowheads: George/O'Hara

Traditional stone points found in whales landed in the 1980's/1990's (supposedly last used in mid 1800's) sparked great interest in the "aging" of bowhead whales. Traditional knowledge of well marked (white scars) whales passed on from generations ("whale lives two human lives") also hinted at long life. Science is now investigating this issue using multiple tools.

Aging of bowhead whales has required morphologic and chemical analyses of body length, baleen length, eye lens amino acid racemization, collagen – histology and glycation products, contaminants, tympanic bullae assessment, and others. Even today "age" is an elusive determination. Body length can be a reasonable surrogate for age in most mysticetes up to the age of sexual maturity (ASM) given that the growth parameters are known. Beyond this age at maturation, many length/age classes overlap particularly for long-lived cetaceans like the bowhead whale. For baleen length and assessing stable C isotopes in baleen Schell et al. (1989) demonstrated that the age of bowhead whales could be estimated by counting oscillations (annual cycles related to migration) of the stable carbon isotopes in the baleen. However, this technique is currently limited to 11-14 yr due to wear at the distal end of the plate.

Determining age by measuring the degree of racemization of aspartic acid in the eye lens has been applied to several species of marine mammals including bowhead whales (Bada et al 1980; Bada et al. 1983; Nerini 1983a,b; George et al., 1999). This technique indicated bowhead whales attain ages older than 100 years and may approach 150-200 years of age (George et al., 1999). This technique is continuing to be used to "age" bowhead whales.

Collagen histology and chemistry may prove useful by assessing fiber types and density, and glycation products (formation of pentosidine). This approach is in the early stages of testing. The basic questions are: Does connective tissue type or density increase with age (decrease in adipose) in blubber? Do specific fiber type amounts or ratios in blubber parallel age? Does fibrosis in other tissues also accumulate with age (i.e., renal fibrosis)?

Some contaminants are well known to accumulate with age in some tissues including the organochlorines in blubber and cadmium in kidney. These may prove useful but will be limited in utility because some organochlorines concentrations increased with length ("age") in males. However, in females the concentrations increased with length (age) until approx. 13 m, those >13 m had lower concentrations than shorter females. Linear regression analyses for renal cadmium (ug/g ww) vs. body length indicated a significant relationship, indicating it may be useful. However, in older animals Cd concentrations may decrease as the renal tissue changes (i.e., the increased fibrosis) and Cd is lost during this degenerative process, a well-known phenomenon in humans.

8. Comparative Populations

Consideration was given to populations or stocks of bowhead and right whales that could be used in comparative studies. Prioritization of studies was given to the North Atlantic right whale population because of the numerous conservation and management issues affecting this species. Comparisons between NARW that utilize the Bay of Fundy summering grounds with those that do not were recommended. Comparisons with other right whale populations should be explored (e.g., North Pacific, Okhotsk Sea).

Currently the BCBS stock of bowheads presents the best balaenid population for comparative studies because of the extensive research already underway, the availability of good information on population trends, strong population growth and minimal impacts from anthropogenic sources. However, sampling of landed whales is non-random. The Eastern Arctic bowhead stocks may also provide a good comparison for the NARW, but the workshop lacked expertise to examine this possibility and research is likely less intense as compared to efforts in northern Alaska (i.e., industry driven concerns).

Comparative studies should extend from the molecular to the cell to the individual to the population levels, and comparison of ocean basin effects on key population parameters were recommended. For example, the NARW population lives in an "urban ocean" while several of the other balaenid populations suffer far less direct, local impact from human activities. Workshop participants pointed out the need to enhance live bowhead whale examination and sampling in the Beaufort Sea to obtain baseline data related to reproductive status, visual and remote biopsy health assessment, and stress in relation to changes in environmental and industrial conditions. Reliance on examination of landed animals will not adequately address some of the questions and concerns in this region. Examination of bowhead whales to assist in primarily NARW projects is already underway.

A life history comparison (Table 2) was constructed to compare the various stocks from a population perspective to place the workshop findings and discussions in context. Also, these apparent differences may better focus population comparisons and information needs.

9. Available Methodologies for Studying Health and Physiology of Living and Landed/Stranded Whales

It was agreed that studies (from a conservation and management perspective) of baleen whale health are important because they may be predictive of reproductive success and survivorship at the population level. Participants identified the key extrinsic and intrinsic factors potentially affecting reproduction and survivorship in right whales and bowheads. These included: 1) habitat/nutrition (encompassing prey quantity/quality, prey competition, oceanographic effects on food source); 2) natural and anthropogenic chemical exposure; 3) disease; 4) industrial activities/disturbance (noise, fishing, shipping); 5) genetics; and, 6) climate change.

This was followed by a discussion of the important population parameters that should be monitored, and the following list was developed:

Population parameters to be monitored:

- Pregnancy rate
- Age at sexual maturity
- Calf production
- Senescence (both sexes)
- Age-specific mortality and serious injury rates
- Population demographics
- Range and dynamic changes therein
- Habitat use patterns
- Body condition indices

Table 3 lists the methodologies available to assess the key factors and population parameters, and the feasibility of applying these measures to both living and dead/stranded right whales and bowheads is indicated in this Table by "+" (feasible), "-" (not possible) or "+/-" (partially feasible).

A list of needed validation and calibration studies for the methodologies in Table 3 is provided in Appendix 1. Some recommendations for Standardization of Methodologies are in Appendix 4. Because there was insufficient time during this workshop to cover the topic rigorously, the participants recommended that a separate workshop be convened to discuss methodology standardization and validation in detail.

The advantages of sampling living whales vs. landed/stranded whales were discussed. Studies of landed/stranded whales allow for better sampling control in terms of location and quantity and detailed sampling of an individual during post-mortem examinations. Stranded whales present the opportunity to address disease processes and diagnostics in moribund animals prior to death. While the information about individuals is in-depth, it only gives a single snapshot in time of health. In contrast, studies of living whales have the advantage of multiple sampling providing much more powerful information on temporal and spatial relationships between measures of health and condition, life history and environmental variables. Additionally, it is possible to assess behavioral endpoints in living whales. Basically, the combination of efforts is the most rewarding.

Workshop participants pointed out the need to enhance live bowhead whale examination and sampling in the Beaufort Sea to obtain baseline data related to reproductive status, visual and remote health assessment, and stress in relation to anticipated changes in environmental and industrial conditions (e.g. increases in fishing effort, shipping activity, seismic studies). Examination of landed bowhead whales to assist in validation and comparative studies of NARW reproduction is already underway and should be expanded to other research areas.

Participants advocated for use of "mysticete-centric oceanography" versus just basic chemical and physical oceanography to look at spatial and temporal relationships between environmental factors and balaenid populations (see presentations by Mayo and Kenney). For example, habitat quality description in parallel with study of whale movements, distribution, and population reproductive performance; prey patch structure and composition could be linked to whale behavior overlaying habitat description/model; evaluation of dynamics of feeding opportunities; prey composition and density on a variety of scales; prey quality and quantity – lipids, elements, vitamins and other components of prey with the goal of describing nutritional and habitat needs.

10. Research Recommendations

Some breakout groups developed testable research hypotheses and identified the populations targeted, the methodology to be applied and current data to be used. Breakout groups addressed four areas of focus: 1. Genetics, 2. Toxicants and disease, 3. Habitat and nutrition, and 4. Environmental factors. The groups selected varying approaches. These are presented below.

10.1. Genetics

Hypothesis 1: *Low-allelic diversity at MHC loci is limiting the potential reproductive capacity of NARW.*

Predictions/Possible Tests

1) The Davis Strait bowhead whale stock (DSBS) has a higher allelic diversity than NARW based on 20 year age at maturity and extended longevity.
2) The BCBS bowhead stock may have lost less allelic variation than either the DSBW or the Hudson BS.
3) Sixteenth century BCBS bowhead whale allelic genetic diversity should be similar to current genetic diversity.
4) The NARW population should have lower allelic genetic diversity than the SARW population.
5) Spontaneous abortion should be higher in the NARW population due to low MHC diversity. (Rosalind "Roz" Rolland; pregnancy detection using fecal progesterone levels vs. calving success).

Hypothesis 2: Sub-stock structure exists within the BCBS bowhead whales*.
* Only relevant if it is a management issue.

1) Prediction:
   Summering Western Chukchi and Eastern Beaufort Seas bowheads will have different mitochondrial haplotypes but the same nuclear DNA profiles. (This is because interbreeding occurs in a common breeding ground; based on the beluga model.)
2) Ice conditions determine the summer distribution of the mitochondrial haplotypes of the BCBS whales.

Hypothesis 3: Low MHC diversity is responsible for higher disease incidence.

Predictions/Possible Tests

1) NARW whales will have higher rates of infectious disease (and associated pathology) and lower MHC diversity than other balaenids with higher MHC diversity.
2) Hypothesis: Further determine the relationship between extant bowhead whale stocks, in particular those in North America**.**

Methods:

Predictions/Possible Tests

1) Eastern North American stocks of bowhead stocks will have different mitochondrial haplotypes but the same nuclear DNA profiles. (DFO is collecting samples and could address this issue).

2) Pleistocene ice distribution can explain current genetic distribution of NA bowhead stocks.
3) Determine relatedness between all five extant bowhead stocks (Okhotsk to North Eastern Atlantic).

10.2. Toxicants and Disease

Hypothesis:

Reproductive success is affected by infectious or non-infectious disease.

Population comparisons:

Compare bowhead vs. right whale – population growth curve and abundance estimates (see Table 1): Or

Compare NARW with SARW calving count and interval and growth curve and abundance estimates (see Table 1).

Should evaluate potential of including Eastern Canadian bowhead and Northwest Pacific right whale populations.

For many health and disease related investigations a classic research experimental design is not well suited (i.e., hypothesis based). Solid baseline, background, or "normal" values (data) are needed for these quantitative and observational types of data so an investigator can assess any deviation from normal and possible impacts and causes of disease. The recent efforts to describe health, scars, etc. are methods being established that will likely form the solid foundation required for these health assessments. It is clear that examination of whales in many cases is biased and this hampers the process of describing the population as a whole from these subsets of samples. Some biases include examination of stranded or hunted animals, assessing animals during a certain time of year and location, data and sample collection priorities, and many others. For these above stated reasons, the "Toxicant and Disease" group did not provide detailed hypotheses and focused on data and samples to collect from more of a baseline or monitoring perspective.

Survivorship (no hypothesis presented)

Long term photo-identification (ID) NARW and SARW females – needs to be tied into health assessment observations and fecal and biopsy health indices measurements. The recent techniques described in the report for grossly assessing health and scarring may have very good prognostic value in identifying cohorts with the highest likelihood of not being resighted (mortality expected). This capability can now be recognized and be used *a priori* in experimental design and monitoring programs. This can be the foundation of testable hypotheses in that a quantitative "condition" score could be tested for utility in predicting reproductive performance (recruitment) and survivorship (two critical measures to assess population performance).

To determine the cause of death the above mentioned techniques need to have standardized protocols for stranded NARW, SARW, and bowhead whale data collection. The states of decomposition vary between these events and regions that provide access to these whales and should be appreciated as a major obstacle. Complete necropsy protocols and nomenclature should be standardized and data centrally and uniformly archived to better establish the database for baseline assessments and monitoring.

Tissue archives should be maintained and shared.

Given the approach of the bowhead population to carrying capacity, and the apparent impact of global climate change on Arctic ice cover, it is strongly recommended that all future bowhead mortalities (strandings) be documented for cause of death, to provide a baseline as industrial and military related stressors inevitably increase. A routine photo-ID and scarring catalog effort should be increased to allow better monitoring of population parameters to allow for early warning of potential downward trends.

10.3. Habitat and Nutrition

General Hypothesis:

> The changes in patterns of habitat use affect the survivorship and reproduction of balaenid whales.

** this is probably a testable hypothesis if such change in use can be defined, measured, and documented

Nutrition: Miller, Marx, Mayo

> **Hypothesis:** Variations in nutrition directly affect the survivorship and reproduction of the North Atlantic right whale.

Notes:

A. Compare NARW and SARW and BOWHEADS.
B. Compare Fundy and non-Fundy NARW's. This comparison will be most important if we establish that reproduction and survivorship are substantially different between the two "subpopulations".
C. If we do establish this BOF/NBOF differences in vitals then we need to determine where the non-Fundy females/whales are.

Table for Habitat and Nutrition Hypotheses: Methods and information gaps for testing the above hypothesis.

	NARWF (Fundy)	NARW2 (NonF)	WA Bowhead	SARW
<u>Nutrition</u>				
Morphometrics	2	0 +	2	2
Blubber thickness	2	0 +	2	2
Lipid Content	1	0 +	2	1
Nutrients	0 +	0 +	?	0 +
In and out gesta	0 +	0 -	2?	0 +
Resp gases/biochem	0 +	0 +	0 -	0 +
Stable isotopes	1	0 +	2	2?
<u>Habitat</u>				
Prey patch dynamics in feeding areas	1?	0 -	2?	0 +
Prey quantity and quality	1	0 -	2?	0 +
Foraging Behavior	1	0 -	1?	0 +
<u>Reproductive Success</u>				
Pregnancy	1	0 +	2	0 +
Calf production	2	2	1	2
Age at Sex Mat	1	1	2	2
Fertility (?)	0 -	0 -	2?	0 -
Endocrinology*	1	0 +	1	0 +
Individual Life Hx	2	2	0 +	1
<u>Health and Condition</u>				
Visual	2	1?	0 +	1
Epid/dermal assays (histo)	0 +	0 +	1?	0 +
Blood based studies	0 +	0 -	?	0 -
Feces	2?	0 +?	1?	0 +

Scale: 0 = data not available, 1 = some information available, 2 = data available, + = qualifier that 1+ represents a range between 1 and 2, and – = the endpoint barely meets the indicated numerical value (0- likely relates to no information and difficult logistically). See Appendix 3 for details of Methods.

10.4. Environmental Factors and Habitat Use Hypotheses

Hypothesis 1. *Variability and trends in balaenid population parameters and habitat use are linked to both regional and broad-scale climatic and oceanographic patterns.*

This hypothesis would involve long-term studies to examine comparable climatic and oceanographic patterns in different habitats and oceans used by right whales and bowhead whales analyzed in relationship to population parameters and habitat use over multiple years. Consistency across ocean basins and populations would suggest strong links, while differences would suggest that other factors are involved. For example, reproductive rates have been linked with large-scale oceanic patterns (NAO, SOI) for several species of birds and pinnipeds in the southern hemisphere, and in NA Right Whales (see Kenney, this report). Although the exact causative factors are unknown, some of these linkages are strong, and this approach merits further examination for other stocks and related species and ocean basins. In addition, there are several examples in the last twenty years of temporary seasonal abandonment of "traditional" right whale habitats in the western North Atlantic. The causes of these shifts in distribution in localized areas are unknown, but some studies suggest that oceanographic features, particularly "upstream" temperature, are primarily responsible.

This hypothesis addresses the questions of ocean basin differences, long-term climate and ice cover changes and the effects of ocean temperature and circulation, ice cover and freshwater inputs on population parameters and local distribution and habitat use patterns. A table listing possible balaenid population comparisons, data needs and the current availability of the data is below (**Table for Environment Hypothesis 1**).

Table for Environment Hypothesis 1. Possible balaenid population comparisons and some data needs (with an indication of current availability) for hypothesis 1.

Data Needed	NARW	SARW	EAB	WAB
Population Estimation	X	X	0	X
Demographics	X	X	0	0
Distribution and habitat use	X	X (winter)	?	X (summer)
Large scale oceanographic data, (SOI,NAO, PDO, AO)	X	X	X	X
Ice cover	n/a	n/a	X	X
Ocean temperature	X	?	?	X (summer)
Circulation patterns	X	0	X	X
Freshwater input	X	?	X	X

NARW=North Atlantic right whale; SARW=South Atlantic right whale; EAB=Eastern Arctic bowheads; WAB=Western Arctic bowheads; X=data existing, 0=data not currently available; SOI=Southern Ocean Index, NAO=North Atlantic Oscillation; PDO= Pacific Decadal Oscillation; AO= Arctic Oscillation.

Hypothesis 2. *Reproductive rates and parameters, and survivorship in balaenids are dependent upon food availability and predictability.*

The premise of this hypothesis is that the quality and availability of food resources will affect reproduction and survivorship in balaenids. Studies are needed to make a direct connection between prey quality and quantity and reproduction and survivorship. This would involve multiple year studies with standardized sampling including quantifiable measures of prey patchiness, biomass, body condition indices (blubber thickness, lipid content) and reproduction and survivorship.

Table for Environment Hypothesis 2. Possible balaenid population comparisons and some data needs (with an indication of current availability) for hypothesis 2.

Data Needs	NARW	SARW	EAB	WAB
Reproductive parameters	X	X	0	X
Body condition (blubber thickness, lipid content)	X	0	0	X
Prey Patchiness	X (Cape Cod Bay)	0	0	0
Biomass availability	0	0	0	0

Hypothesis 3. *Human activities (including shipping, fishing, noise, seismic exploration, dredging, coastal effluents etc) will alter balaenid habitat use patterns, and secondarily impact reproduction and survivorship.*

Some evidence exists for the displacement of gray whales and bowheads from seismic activity. However, there are no studies on the effects of such displacement on reproduction and survivorship. Studies are needed to identify baseline habitat use patterns, quantify human activities, and develop analyses for determining effects beyond the behavioral level. The rationale for population comparisons is based upon varying intensity of human activities in different habitats and ocean basins. For instance, North Atlantic right whales are subjected to high levels of impacts from fishing and shipping activities compared to South Atlantic right whales, Eastern and Western Arctic bowheads. Population comparisons and data needs for these studies are given below in **Table for Environment Hypothesis 3**.

Table for Environment Hypothesis 3. Possible balaenid population comparisons and some data needs (with an indication of current availability) for hypothesis 3.

Data Needs	NARW	SARW	EAB	WAB
Regional/seasonal habitat use patterns	X	Winters only	Summers only	Spring, Summer, Fall
Before, during after data on human activities	Some	Some	0	X
Reproductive parameters	X	X	0	X

Conclusion: See Executive Summary.

Acknowledgements:

This workshop was possible because of the dedication of many who are concerned about the health status and abundance of large baleen whales; this includes the participants who donated their time. Funds were provided by the Marine Mammal Stranding and Health

Program (OPR of NMFS, Silver Spring, MD via Dr. Teri Rowles), and the North Slope Borough. Kraus and Rolland arranged local logistics support for the meeting and assured quality accommodations and food while in Boston (this includes personal space for the "pile of Alaskans" that were so graciously supported). The Secretariat of the International Whaling Commission allowed Dr. Greg Donovan the time to Chair our meeting, for that we are very grateful.

Appendix 1: Recommended validation and calibration studies of methodologies listed in Table 3.

Methods	Validation/calibration needed
Nutrition	
Detailed Morphometrics	1) Develop standard landmarks. 2) Assess effects of posture and stretching; what are limitations of comparing live whales v. landed/stranded can we develop length & girth specific correction factors for free ranging v. "stretched" landed/stranded whales? 3) Take multiple length and girth measures in water and out of water in varying positions to address potential variations.
Photogrammetry	1) Opportunistic comparisons of measurements on landed/stranded with photogrammetry of living whales. Needed for ground truthing measurements as well as effects of hauling out (whale stretching).
Blubber thickness	1) Develop specific landmarks and record multiple measures (strata) from >2 predetermined sites on and off the whale. Are these measurements significantly different? 2) Calibrate ultrasound measures with physical measure (ruler) of blubber on fresh landed/stranded whales including full thickness "dart" measurement (P. Best's dart).
Lipid Content	1) Multiple analytical techniques require quantitative comparison – what is actually measured by each method requires determination. 2) *Compare lipid content derived from remote biopsy to full thickness and stratified sampling. 3) Examine effects of age, gender, and reproductive state using tissues from landed whales. 4) Determine post-mortem changes in types and concentrations of lipid classes and fatty acids over time (hours to days) under temperate ambient conditions. 5) Determine effects of storage, preservation method, and archiving on various lipid measures over time.
Nutrients (vitamin, elements, essential fatty acids, etc.)	1) Multiple techniques require quantitative comparison – what is actually measured? Caution in comparing data from different methods should be stressed. 2) Compare remote biopsy procedures to full thickness and stratified sampling 3) Examine effects of age, gender, and reproductive state using tissues from landed whales. 4) Determine post-mortem changes in types and concentrations of nutrient over time (hours to days) under temperate ambient conditions. 5) Determine effects of storage, preservation method, and archiving on measures over time. 6) Compare minerals in epidermis, and lipophilic vitamins and fatty acids in dermis from biopsies to full thickness samples and other tissues (blood, liver, etc.).
In and out gesta (consists of: 1. Free swimming prey- 2. Stomach contents- 3. Feces from GI tract 4. Free floating feces-	For most of the 4 categories: 1) Prey identification 2) Nutritional analyses (calories, % lipid, nutrients) 3) Parasitology (species, prevalence, compare dead whales (burden) to free ranging fecal sample to develop extrapolation model). 4) Steroid hormone analyses (estrogen, progestins, androgens, glucocorticoids) consider interfering compounds, post mortem time effects, compare to reproductive status and hormone levels in blood. 5) Compare sample types in describing feeding quality and quantity.
Respiratory gases/biochemistry	1) New technique- requires significant validation studies before it can be applied.
Stable isotopes	1) Compare matrices and be cautious of inter-laboratory differences. 2) Calibrate epidermis with known feeding ecology and other matrices (like blood, liver, muscle and baleen).

Methods	Validation/calibration needed
Reproductive Success	
Pregnancy	1) Blubber sex steroids: concentrations need to be compared to life history data (cow sampled and produces a calf later), and status of landed and stranded whales for validation of pregnancy predictability. 2) Feces: fecal steroids already validated for NARW and BCBS bowheads. Progesterone accurately predicts pregnancy in both species. Comparison between fecal and serum progesterone levels in bowheads is underway (Rolland et al.). 3) Gross morphology and histology of reproductive tracts-effects of autolysis on organ appearance (for landed stranded) should be assessed.
Health and Condition	
Morphometrics	see above
Photogrammetry	see above
Blubber (EDHl) thickness	see above
Lipid Content	see above
Nutrients	see above
In and out gesta	see above
Respiratory pathogens/microflora	1) Development of proper sampling techniques and knowledge of “normal’ flora is needed.
Stable isotopes	see above
General Histo/histopathology*	1) Assess post mortem changes over time and effects of the degree of autolysis in health assessment and diagnostics. 2) Develop an image archive for “baseline” data use as well as diagnostics.
Toxicants (natural and anthropogenic)	1) Standardize dermal (blubber) site and depth of sampling effects, post-mortem time effects, etc. refer to Pollution IWC 2000+ (Reijnders et al. 1999). 2) Analyze for non-traditional contaminants such as the butyltins, brominated compounds, phthalates, and alkylphenols 3) Laboratories MUST participate in interlaboratory comparisons and use similar marine mammal based standards and reference materials.

Appendix 2: 2003 Bowhead/Right Whale Comparative Health and Physiology Workshop

"Bowhead/Right Whale Comparative Health and Physiology Workshop"

March 25-26, 2003
New England Aquarium, Boston, MA

Workshop Goal

To determine the measurable physical, chemical, biochemical and physiological parameters that would be used to assess the health of bowhead whales and right whales, and to establish priorities for comparative investigations of mysticete nutritional and health status.

Three Workshop Objectives:

1. Review ongoing studies, identify new areas for collaborative research and develop specific hypothesis driven comparative studies of right whales and bowheads to assess health, physiology and nutritional status.
2. Develop long-term standardization of sampling and data collection to allow comparisons.
3. Assess the rationale of the bowhead/right whale comparative model for different research areas.

AGENDA

Day 1 Tuesday March 25th

I. Ice Breaking and Introductions

830AM Coffee and pastries (provided)
900AM Welcome by Scott Kraus, NEAq Director of Research
905AM Introductions led by Chair
915AM Review of Objectives/Goals- Willetto and Rolland

II. Species Overview

930AM-1015AM: 15 minute presentations

A. **Alaska bowheads: The Health & Physiology Workshop Report and Overall Status**- Willetto/George
B. **Status of eastern Arctic bowheads: Research and Management Summary-** Cosens
C. **Status of North Atlantic right whales: Research and Management Summary-** Kraus

1015AM Break

III) Feeding Ecology, Nutrition, Blubber Studies: Relationship to Health and Reproduction

1030-1100AM: Bowhead Current and Future Research
[Feeding ecology overview of bowhead whales by O'Hara and George]

1. LGL feeding study (population and individual)
2. Trophic ecology (Hoekstra et al. stable isotopes and contaminants)
3. Blubber characteristics- (Workshop & CIFAR Progress reports)
4. Nutrition – (nonblubber) essential elements; muscle mass, histology and biochemistry; digestive organ histology; nutritive value for consumers.

1100AM-1145: Right Whale Feeding Ecology Current and Future Efforts
1115AM: Blubber Thickness Studies and Reproduction-Moore
1130AM: Linking Food Resources to Reproduction-Mayo
1145AM: Linking Climate (NAO) to Reproduction-Kenney

1145-1200: Discussion and Questions
Noon-1PM: Lunch (provided)

III. Nutrition/Blubber Studies/Feeding Ecology (cont'd)

100PM-230PM: Discussion led by Chair

Discussion points:

1. Methodology validation (*in situ* and *in vitro*)
2. Within animal variability of blubber measurements based on physically measured sites and selection of best indicator sites
3. What is blubber anyway? Standardization in consideration of:
   Epidermis + dermis = blubber
   Above + hypodermis = blubber
4. Lipid measurements
5. Fatty acid profiles
6. Stable isotopes
7. Field studies on prey species (nutrition, climate changes)
8. Collaborative research-ideas and hypotheses

230PM: Break

IV. Genetics

245PM- 330PM
245AM –315PM: Review of existing research by Dr. White
315-330 PM: Review of bowhead genetic studies O'Hara/George
330-430PM: Discussion

Discussion points

1. MHC diversity questions that may apply to both bowhead whales and right whales.
2. Collaborative research ideas and hypotheses.

430-500PM: Discussion and wrap-up
Nutrition and genetics wrap up
Chair's Comments on Today and Preparation for Wednesday.
0530: Dutch Dinner at Legal Seafoods or on your own

2 Wednesday March 26th

830AM-900AM: Coffee and pastries

V. Reproduction and Stress

900AM-930AM: Calf production indices for assessing reproductive "health"
0900: Right whales (Scott Kraus)
Aerial surveys/photo ID
0915: Bowhead whales (George/O'Hara)
Ice based census
Examination of harvested whales
Aerial surveys/photo ID

930-0950AM Sex and Stress Hormones
Overview of ongoing work in reproductive/stress hormones in right whales and bowheads- Rolland.
10 min questions
1000AM: Break (15 minutes)
1015-1130: DISCUSSION

Discussion Points:

1. Stress corticoids and heat shock proteins, if pursued how will we validate? Validate with the bowhead model? Location on body effects of heat shock measures – conduct research in bowhead whales?
2. Measures of reproductive health – ocean basin comparisons.

3. Measures of reproductive health – interbirth intervals, non-calving adults, ASM, rate of spontaneous abortions or neonatal mortalities, etc. Can we measure stress as a factor in these things?
4. Identify potential environmental stressors e.g. ship collisions, noise, oil, inadequate nutrition etc., and identify or develop methods to evaluate effects.
5. Where can assessing reproductive status in free ranging whales take bowhead and right whale research teams?
6. Where can assessing stress status in free ranging whales take bowhead and right whale research teams?
7. Behavior related to stress? Surface times, breathing patterns, "skittishness", epidermal changes, etc. (reliability, significance, stability over time)

VI. Contaminants & Toxicants (30 minutes)

1130-Noon: Discussion only

Discussion points:

1. Assessing effects using cell lines? Invite John Wise?
2. Contaminant bioactivity
3. Non-traditional contaminants (phthalates, tributyltins, etc.)
4. Hydrocarbons-acute/chronic, baseline levels of exposure/effects

    Michael Moore and Rosalind Rolland for NARW, for bowheads Teri Rowles, Carla Willetto and Todd O'Hara.

Noon-100PM: lunch (provided)

VII. Pathology/Disease and Health Assessments

100PM-0300PM

100-120PM: Visual Health Assessment in Right Whales-Pettis

120-140PM: Scarring Analysis of Right Whales- Knowlton

140-200 PM: Bowhead Skin Studies: O'Hara and Willetto

200-220PM: Right Whale Skin Lesion Analysis: Marx

220-300PM: Discuss possible comparative studies of pathology and skin lesions, as well as other infectious disease possibilities

300PM: break (15 minutes)

VIII. Other Possible Areas of Consideration for Short Discussion

315-400PM

315-330: Marine Biotoxins- PSP studies in right whales- proposed bowhead work: Rolland

330-345: Aging Studies In bowheads: George/O'Hara addressing:

Body length
Baleen length
Eye lens – aspartic acid racemization
Collagen histology and chemistry
Contaminants (i.e. renal Cd)
Genetic

X. Final Discussion and Wrap-up

345-500PM

Discussion Points

Next Steps – Likely comparative studies
Potential collaborations
Workshop Report

500PM: Adjourn

Appendix 3: List of Participants

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Appendix 4: Selected Recommendations for Right Whale/Bowhead Standardization of Methodologies and Data Collection

General Recommendations:

- Hold a workshop on methodology.
- A major goal is to utilize bowheads to validate and calibrate assays that can be applied to living right whales (analyses applied to fecal and biopsy samples, blubber thickness measurements, visual health assessment)
- Standardize location of remote biopsy sampling.
- Calibrate biopsy samples with whole thickness blubber studies
- Determine post mortem time effect on various samples from landed/stranded whales.
- Develop methods to take blood samples from entangled right whales.
- Standardize methods for collecting habitat data, photo-identification techniques and visual health assessment methods.
- Conduct studies of inner ears of bowhead whales to compare with studies on NARW inner ears (by D. Ketten)
- NARW necropsy protocol should use the bowhead gonad morphology data sheet. In both species, measurements of snout to genital slit center should be taken. Data quality scores should be assigned to morphometric data.
- Investigate feasibility of shipboard studies of free-ranging bowheads. Non-invasive studies would include; photo-identification, prey-based studies, visual health assessment, PSP toxin levels in prey and feces, biopsies, and steroid hormones in feces.
- A high priority should be given to collecting baseline data on living bowhead whales for future use to assess impacts of expected increases in industrial activities.