# Health Assessments of North Atlantic Right Whales

## **Bibliography**

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### **Table of Contents**

Background & Scope	3
Sources Reviewed	
Section I: Assessment Methods	4
Section II: Health Metrics in Right Whales	10
Section III: Health Assessments of Other Marine Mammals	19
Section IV: Organizations Doing Health Assessments	25
Section V: Peripheral Materials	26

#### **Background & Scope**

A working group as part of the Office of Protected Resources within NOAA Fisheries is striving to improve its knowledge of North Atlantic right whale (NARW) health to advance species recovery. The NARW is an endangered species protected under the Endangered Species Act. To that end, this bibliography was developed to give a comprehensive overview of literature related to NARW health indicators and the types of health assessments being done with other marine mammals.

#### Section I – Assessment Methods

This section covers different methods currently being used to assess marine mammal health in living and deceased animals. These include methods to measure weight, hormone levels, body condition, nutritional status, disease, and other health indicators.

#### Section II - Health Metrics in Right Whales

This section covers research being done into metrics which could help establish the health and well-being of right whales, including North Atlantic right whales.

#### Section III – Health Assessments of Other Marine Mammals

This section covers the types and methods of health assessments currently being used with marine mammals that are not right whales. It includes health assessments of animals such as humpback whales, Hawaiian monk seals, bottlenose dolphins, manatees, and porpoises.

#### Section IV – Organizations Doing Health Assessments

This section covers three international organizations that are currently working on health assessments of marine mammals and provides links to their websites and working documents.

#### Section IV - Peripheral Materials

This section covers materials which are not directly related to the topic but may still be of interest or use. It includes research into toxicity in right whales, effects of climate change on whale health, and health metrics in marine mammals such as bowhead whales.

#### **Sources Reviewed**

Along with a web search for news items and other relevant materials the following databases were used to identify sources: Clarivate Analytics' Web of Science: Science Citation Index Expanded, EBSCO Academic Search Complete, Nexis.com, ProQuest's Aquatic Sciences and Fisheries Abstracts, BioOne, and JSTOR. Only English language materials were included. There was no date range specification in order to cover any relevant research, although priority was given to publications in the last twenty years.

#### **Section I: Assessment Methods**

Barratclough, A., et al. (2014). "How much does a swimming, underweight, entangled right whale (Eubalaena glacialis) weigh? Calculating the weight at sea, to facilitate accurate dosing of sedatives to enable disentanglement." *Marine Mammal Science* 30(4): 1589-1599. <a href="https://doi.org/10.1111/mms.12132">https://doi.org/10.1111/mms.12132</a>

Producing a simple, accurate method of establishing a body weight will facilitate more accurate sedative dosing, reducing the associated risks. Improving techniques will encourage increased use of sedation to assist disentangling if the whale is identified early, rather than intervening when the whale has already deteriorated too far. North Atlantic right whales are the least tractable species to disentangle due to their persistent and successful avoidance of close-approaching vessels (Moore et al. 2010). Due to their increased muscle strength compared to other species right whales have been shown to be less tolerant to the additional drag placed upon them during a disentanglement effort (Johnson et al. 2005). Effective sedation methods are therefore even more applicable to this species to facilitate approach. However, no matter how well the disentanglement process is developed, the only lasting solution to the entanglement problem is avoidance of entanglement in the first place.

Burgess, E. A., et al. (2018). "Quantifying hormones in exhaled breath for physiological assessment of large whales at sea." *Scientific Reports* 8: 14. <a href="https://doi.org/10.1038/s41598-018-28200-8">https://doi.org/10.1038/s41598-018-28200-8</a>

Exhaled breath analysis is a non-invasive assessment tool that has shown promise in human diagnostics, and could greatly benefit research, management, and conservation of large whales. However, hormone assessment of whale respiratory vapor (blow) has been challenged by variable water content and unknown total volume of collected samples. To advance this technique, we investigated urea (a compound present in narrow range in circulation) as a normalizing factor to correct for blow sample concentration. Normalized progesterone, testosterone, and cortisol concentrations of 100 blow samples from 46 photo-identified North Atlantic right whales (Eubalaena glacialis) were more biologically relevant compared to absolute estimates, varying by sex, age class, or individual. Progesterone was elevated in adult females compared with other cohorts and highest in one independently confirmed pregnant female. For both sexes, testosterone was two-fold higher in reproductively mature whales but studied adult females showed the widest variation. Cortisol was present in relatively low concentrations in blow and demonstrated variation between individual whales, suggesting potential for studies of individual differences in adrenal activity. Incorporation of methodologies that normalize sample concentration are essential for blow hormone analysis of free-swimming whales, and measurement of urea could be used to optimize non-invasive physiological assessment of whales.

Christiansen, F., et al. (2016). "Noninvasive unmanned aerial vehicle provides estimates of the energetic cost of reproduction in humpback whales." *Ecosphere* 7(10): 18. <a href="https://doi.org/10.1002/ecs2.1468">https://doi.org/10.1002/ecs2.1468</a>

An animal's body condition will affect its survival and reproductive success, which influences population dynamics. Despite its importance, relatively little is known about the body condition of large whales and its relationship to reproduction. We assessed the body condition of humpback whales (Megaptera novaeangliae) at a breeding/resting ground from aerial photographs recorded using an unmanned aerial vehicle (UAV). Photogrammetry methods were used to measure the

surface area of individual whales, which was used as an index for body condition. Repeated measurements of the same individuals were not possible; hence, this study represents a crosssectional sample of the population. Intraseasonal changes in the body condition of four reproductive classes (calves, immature, mature, and lactating) were investigated to infer the relative energetic cost that each class faces during the breeding season. To better understand the costs of reproduction, we investigated the relationship between female body condition (FBC) and the linear growth and body condition of their dependent calves (CBC). We documented a linear decline in the body condition of mature whales (0.027 m(2)/d; n = 20) and lactating females (0.032 m(2)/d; n = 20)m(2)/d; n = 31) throughout the breeding season, while there was no change in body condition of immature whales (n = 51) and calves (n = 32). The significant decline in mature and lactating female's body condition implies substantial energetic costs for these reproductive classes. In support of this, we found a positive linear relationship between FBC and CBC. This suggests that females in poorer body condition may not have sufficient energy stores to invest as much energy into their offspring as better conditioned females without jeopardizing their own body condition and survival probability. Measurement precision was investigated from repeated measurements of the same animals both from the same and different photographs, and by looking at residual errors in relation to the positioning of the whales in the photographs. The resulting errors were included in a sensitivity analysis to demonstrate that model parameters were robust to measurement errors. Our findings provide strong support for the use of UAVs as a noninvasive tool to measure the body condition of whales and other mammals.

Durban, J. W., et al. (2016). "Photogrammetry of blue whales with an unmanned hexacopter." *Marine Mammal Science* 32(4): 1510-1515. <a href="https://doi.org/10.1111/mms.12328">https://doi.org/10.1111/mms.12328</a>

Baleen whales are the largest animals ever to live on earth, and many populations were hunted close to extinction in the 20th century (Clapham et al. 1999). Their recovery is now a key international conservation goal, and they are important in marine ecosystems as massive consumers that can promote primary production through nutrient cycling (Roman et al. 2014). However, although abundance has been assessed to monitor the recovery of some large whale populations (e.g., Barlow et al. 2011, Laake et al. 2012) many populations are wide-ranging and pelagic, and this inaccessibility has generally impeded quantitative assessments of recovery (Peel et al. 2015).

To augment traditional abundance monitoring, we suggest that photogrammetric measures of individual growth and body condition can also inform about population status, enabling assessment of individual health as well as population numbers. Photogrammetry from manned aircraft has used photographs taken from directly above whales to estimate individual lengths (Gilpatrick and Perryman 2008) and monitor growth trends (Fearnbach et al. 2011), and shape profiles can be measured to assess body condition to infer reproductive and nutritional status (e.g., Perryman and Lynn 2002, Miller et al. 2012). Recently, Durban et al. (2015) demonstrated the utility of an unmanned hexacopter for collecting aerial photogrammetry images of killer whales (Orcinus orca); this provided a noninvasive, cost-effective, and safe platform that could be deployed from a boat to obtain vertical images of whales. Here we describe the use of this small, unmanned aerial system (UAS) to measure length and condition of blue whales (Balaenoptera musculus), the largest of all whales.

Fearnbach, H., et al. (2018). "Using aerial photogrammetry to detect changes in body condition of endangered southern resident killer whales." *Endangered Species Research* 35: 175-180. <a href="https://doi.org/10.3354/esr00883">https://doi.org/10.3354/esr00883</a>

The endangered population of southern resident killer whales Orcinus orca is hypothesized to be food-limited, but uncertainty remains over if and when the availability of their primary prey, Chinook salmon Oncorhynchus tshawytscha, is low enough to cause nutritional stress. To measure changes in body condition, we collected 1635 measurable images from a helicopter hovering 230-460 m above whales, and linked these to individuals with distinctive natural markings. Head width (HW), measured at 15% of the distance between the blowhole and the dorsal fin (BHDF), was measured from images of 59 individuals in 2008 (from a population of 84) and 66/81 individuals in 2013, enabling assessment of between-year changes for 44 individuals (26 females, 18 males). Of these, 11 had significant declines in the ratio of HW/BHDF compared to 5 with significant increases. Two whales with declines died shortly after being photographed, suggesting a link between body condition and mortality. Most (8/11) of the significant declines in condition were from 1 social pod (I-pod), and all the whales that increased in condition were from one of the other 2 pods, K-pod (n = 3) and L-pod (n = 2). Notably, 11/16 whales that changed condition were re productive-aged females and there were no adult males with significant changes. This likely reflects the increased energetic costs of lactation to reproductive females, and the nutritional help provided to adult males through prey sharing. These data demonstrate the utility of aerial photo-grammetry as a noninvasive approach for providing quantitative data on body condition, and support monitoring the condition of reproductive females as key indicators of nutritional stress.

Hall, A. J., et al. (2010). "Epidemiology, disease, and health assessment." *Marine Mammal Ecology and Conservation: A Handbook of Techniques.* I. L. Boyd, W. D. Bowen and S. J. Iverson, Oxford University Press: 144-163.

Understanding marine mammal health and disease and the related impacts on populations is crucial to support effective conservation and management decisions. However, ethical issues involved in conducting experimental studies can limit the scope of marine mammal health research. This forces a focus and reliance on epidemiological studies, similar to those that have been applied to studying factors affecting human health. Marine mammal epidemiology is additionally challenging because most marine mammals are not easily observed for most of their lives, disease states are generally difficult to detect, and reporting mechanisms for disease used in human and veterinary epidemiology (i.e. birth, death, and disease records) are virtually non-existent for marine mammals. Nonetheless, despite these drawbacks, there are many ways in which robust and reliable epidemiological studies can be applied in the field of marine mammal science.

Hunt, K. E., et al. (2013). "Overcoming the challenges of studying conservation physiology in large whales: a review of available methods." *Conservation Physiology* 1(1): 24. <a href="https://doi.org/10.1093/conphys/cot006">https://doi.org/10.1093/conphys/cot006</a>

Large whales are subjected to a variety of conservation pressures that could be better monitored and managed if physiological information could be gathered readily from free-swimming whales. However, traditional approaches to studying physiology have been impractical for large whales, because there is no routine method for capture of the largest species and there is presently no practical method of obtaining blood samples from free-swimming whales. We review the currently available techniques for gathering physiological information on large whales using a variety of non-

lethal and minimally invasive (or non-invasive) sample matrices. We focus on methods that should produce information relevant to conservation physiology, e.g. measures relevant to stress physiology, reproductive status, nutritional status, immune response, health, and disease. The following four types of samples are discussed: faecal samples, respiratory samples ('blow'), skin/blubber samples, and photographs. Faecal samples have historically been used for diet analysis but increasingly are also used for hormonal analyses, as well as for assessment of exposure to toxins, pollutants, and parasites. Blow samples contain many hormones as well as respiratory microbes, a diverse array of metabolites, and a variety of immune-related substances. Biopsy dart samples are widely used for genetic, contaminant, and fatty-acid analyses and are now being used for endocrine studies along with proteomic and transcriptomic approaches. Photographic analyses have benefited from recently developed quantitative techniques allowing assessment of skin condition, ectoparasite load, and nutritional status, along with wounds and scars from ship strikes and fishing gear entanglement. Field application of these techniques has the potential to improve our understanding of the physiology of large whales greatly, better enabling assessment of the relative impacts of many anthropogenic and ecological pressures.

Hunt, K. E., et al. (2014). "Detection of steroid and thyroid hormones via immunoassay of North Atlantic right whale (Eubalaena glacialis) respiratory vapor." *Marine Mammal Science* 30(2): 796-809. https://doi.org/10.1111/mms.12073

Steroid and thyroid hormone analyses can potentially provide valuable information about many aspects of physiology in marine mammals, including stress responses, reproductive maturity, reproductive cyclicity, pregnancy status, and metabolic rate (Rolland et al. 2005; Hunt et al. 2006, 2013; Biancani et al. 2009; Wasser et al. 2010; Kusuda et al. 2011; Ayres et al. 2012). However, it has been difficult to collect the relevant biological samples from living, large cetaceans for endocrine studies (Rolland et al. 2007, Amaral 2010). Analysis of fecal hormone metabolites has proved quite biologically informative (Rolland et al. 2005, 2012; Hunt et al. 2006), but feces can only be collected opportunistically. Blubber hormone analysis shows promise but is somewhat invasive, permitting issues can restrict repeated sampling of individuals, and the pharmacokinetics of blubber hormone turnover and timeframe of deposition are still uncertain (Mansour et al. 2002; Keller et al. 2006, 2009; Perez et al. 2011; Hunt et al. 2013). A novel method that has been receiving increasing attention is analysis of hormones in condensed droplets of respiratory vapor ("blow"). Large whales usually blow several times at the surface after extended dives, and previous research has demonstrated that blow droplets can be collected successfully from free-swimming large whales using pole-based methods or remote-controlled helicopters (Hogg et al. 2005, 2009; Acevedo-Whitehouse 2010). Hogg et al. (2009) reported detectable testosterone and progesterone in blow samples collected from North Atlantic right whales (Eubalaena glacialis, NARW) and humpback whales (Megaptera novaeangliae) using liquid chromatography-mass spectrometry (LC-MS). However, LC-MS is relatively expensive, requires specialized equipment, and is not widely available to many researchers. Further, mass spectrometry can be vulnerable to hormone degradation, e.g., if a hormone is chemically modified it may not be recognized (Wood et al. 2008, Hogg et al. 2009).

Krause, D. J., et al. (2017). "An accurate and adaptable photogrammetric approach for estimating the mass and body condition of pinnipeds using an unmanned aerial system." *Plos One* 12(11): 20. https://doi.org/10.1371/journal.pone.0187465

Measurements of body size and mass are fundamental to pinniped population management and research. Manual measurements tend to be accurate but are invasive and logistically challenging to obtain. Ground-based photogrammetric techniques are less invasive, but inherent limitations make them impractical for many field applications. The recent proliferation of unmanned aerial systems (UAS) in wildlife monitoring has provided a promising new platform for the photogrammetry of free-ranging pinnipeds. Leopard seals (Hydrurga leptonyx) are an apex predator in coastal Antarctica whose body condition could be a valuable indicator of ecosystem health. We aerially surveyed leopard seals of known body size and mass to test the precision and accuracy of photogrammetry from a small UAS. Flights were conducted in January and February of 2013 and 2014 and 50 photogrammetric samples were obtained from 15 unrestrained seals. UAS-derived measurements of standard length were accurate to within 2.01 +/- 1.06%, and paired comparisons with ground measurements were statistically indistinguishable. An allometric linear mixed effects model predicted leopard seal mass within 19.40 kg (4.4% error for a 440 kg seal). Photogrammetric measurements from a single, vertical image obtained using UAS provide a noninvasive approach for estimating the mass and body condition of pinnipeds that may be widely applicable.

Perryman, W. L. and M. S. Lynn (2002). "Evaluation of nutritive condition and reproductive status of migrating gray whales (Eschrichtius robustus) based on analysis of photogrammetric data." Journal of Cetacean Research and Management 4(2): 155-164.

<a href="https://swfsc.noaa.gov/uploadedFiles/Divisions/PRD/Programs/Photogrammetry/Perryman">https://swfsc.noaa.gov/uploadedFiles/Divisions/PRD/Programs/Photogrammetry/Perryman and Lynn 2002 Gray Whale JCRM.pdf</a>

Vertical aerial photographs were collected of gray whales migrating along the California Coast between 1994 and 1998 to readdress some published findings on the biology and life history of this population based on examination of specimens. For each whale, an attempt was made to measure standard total length, the width of the whale at its widest point, the distance from the tip of the rostrum to the widest point, and the width of the flukes. For southbound gray whales, early migrants were longer on average and more likely to be parturient than those migrating later. Nearterm pregnant females were wider relative to their length than other southbound gray whales. This difference was easily detected by visual inspection of the images and through statistical evaluation of length and width data. There was 100% agreement between identification of parturient females based on linear regression analysis of length and width and discriminate analysis of all measurements. Based on the proportion of parturient females to those with calves during sampling of southbound whales, the median calving date was estimated to be 13 January. Southbound calves averaged 4.6m in length; those photographed northbound in late April, at an age of about three months, averaged 7.1m. Average length for yearlings, based on combined southbound and northbound data, was 8.5m. Residuals from a regression of width on length were compared, and significant changes in the relationship were detected which were consistent with changes in nutritive condition or fatness described from examination of whales taken along the California Coast between 1959 and 1969 (Rice and Wolman, 1971). Parturient females were the widest relative to their length and northbound cows with calves were the narrowest in the sample. The relationship between length and width for migrating gray whales that were not parturient or associated with a calf, showed that southbound gray whales were significantly wider than northbound whales photographed approximately 60 days later. These results indicate that the predictable but relatively small changes in condition or fatness of gray whales associated with

fasting during their winter migration can be reliably detected in measurements from vertical aerial photographs.

Pettis, H. M., et al. (2004). "Visual health assessment of North Atlantic right whales (Eubalaena glacialis) using photographs." *Canadian Journal of Zoology* 82(1): 8-19. https://doi.org/10.1139/z03-207

Although trends in reproduction, mortality, and entanglement events have been analyzed for the endangered North Atlantic right whale (Eubalaena glacialis) population, no method has been available to assess individual right whale health. Here, we describe a technique for assessing health based on evaluation of selected physical parameters from archived photographs of right whales. A scoring system was developed to assess body and skin condition, blowhole cyamids, and rake marks in over 200 000 photographs. Comparison of body condition scores of females during calving and noncalving years found that females were significantly thinner in calving years and in the year after calving compared with the year before calving, showing that changes in body condition known to occur during the reproductive cycle can be successfully evaluated from photographs. Comparison of scores for all parameters between living whales and whales with more than a 5-year gap in sighting history ("presumed dead") found that presumed dead whales received health assessment scores indicating compromised health with body condition emerging as a key visual indicator. This health assessment method provides a new tool to monitor health trends in right whales at individual and population levels and may provide a model for assessments of other well-photographed cetaceans.

Schick, R. S., et al. (2013). "Using hierarchical Bayes to understand movement, health, and survival in the endangered North Atlantic right whale." *Plos One* 8(6). <a href="https://doi.org/10.1371/journal.pone.0064166">https://doi.org/10.1371/journal.pone.0064166</a>

Body condition is an indicator of health, and it plays a key role in many vital processes for mammalian species. While evidence of individual body condition can be obtained, these observations provide just brief glimpses into the health state of the animal. An analytical framework is needed for understanding how health of animals changes over space and time. Through knowledge of individual health we can better understand the status of populations. This is particularly important in endangered species, where the consequences of disruption of critical biological functions can push groups of animals rapidly toward extinction. Here we built a statespace model that provides estimates of movement, health, and survival. We assimilated 30+ years of photographic evidence of body condition and three additional visual health parameters in individual North Atlantic right whales, together with survey data, to infer the true health status as it changes over space and time. We also included the effect of reproductive status and entanglement status on health. At the population level, we estimated differential movement patterns in males and females. At the individual level, we estimated the likely animal locations each month. We estimated the relationship between observed and latent health status. Observations of body condition, skin condition, cyamid infestation on the blowholes, and rake marks all provided measures of the true underlying health. The resulting time series of individual health highlight both normal variations in health status and how anthropogenic stressors can affect the health and, ultimately, the survival of individuals. This modeling approach provides information for monitoring of health in right whales, as well as a framework for integrating observational data at the level of individuals up through the health status of the population. This framework can be broadly applied to a variety of systems terrestrial and marine - where sporadic observations of individuals exist.

Schick, R. S., et al. (2016). "Effects of Model Formulation on Estimates of Health in Individual Right Whales (Eubalaena glacialis)." *Effects of Noise on Aquatic Life II*. A. N. Popper and A. Hawkins. Berlin, Springer-Verlag Berlin. 875: 977-985.

Right whales are vulnerable to many sources of anthropogenic disturbance including ship strikes, entanglement with fishing gear, and anthropogenic noise. The effect of these factors on individual health is unclear. A statistical model using photographic evidence of health was recently built to infer the true or hidden health of individual right whales. However, two important prior assumptions about the role of missing data and unexplained variance on the estimates were not previously assessed. Here we tested these factors by varying prior assumptions and model formulation. We found sensitivity to each assumption and used the output to make guidelines on future model formulation.

#### **Section II: Health Metrics in Right Whales**

(2010). *The Urban Whale: North Atlantic Right Whales at the Crossroads*. Cambridge, MA, Harvard University Press.

In 1980 a group of scientists censusing marine mammals in the Bay of Fundy was astonished at the sight of 25 right whales. It was, one scientist later recalled, "like finding a brontosaurus in the backyard." Until that time, scientists believed the North Atlantic right whale was extinct or nearly so. The sightings electrified the research community, spurring a quarter century of exploration, which is documented here.

The authors present our current knowledge about the biology and plight of right whales, including their reproduction, feeding, genetics, and endocrinology, as well as fatal run-ins with ships and fishing gear. Employing individual identifications, acoustics, and population models, Scott Kraus, Rosalind Rolland, and their colleagues present a vivid history of this animal, from a once commercially hunted commodity to today's life-threatening challenges of urban waters.

Hunted for nearly a millennium, right whales are now being killed by the ocean commerce that supports our modern way of life. This book offers hope for the eventual salvation of this great whale.

Burgess, E. A., et al. (2017). "Adrenal responses of large whales: Integrating fecal aldosterone as a complementary biomarker to glucocorticoids." *General and Comparative Endocrinology* 252: 103-110. <a href="https://doi.org/10.1016/j.ygcen.2017.07.026">https://doi.org/10.1016/j.ygcen.2017.07.026</a>

Until now, physiological stress assessment of large whales has predominantly focused on adrenal glucocorticoid (GC) measures. Elevated GC concentrations in feces (fGC) are known to reflect stressful disturbances, such as fishing gear entanglement and human-generated underwater noise, in North Atlantic right whales (Eubalaena glacialis). However, there can be considerable variation in GC production as a function of sex and life history stage, which may confound the interpretation of fGC levels. Additionally, GC antibodies used in immunoassays can cross-react with other fecal

metabolites (i.e., non-target steroids), potentially influencing fGC data. Here, aldosterone concentrations (fALD; aldosterone and related metabolites) were measured in fecal samples from right whales (total n = 315 samples), including samples from identified individuals of known life history (n = 82 individual whales), to evaluate its utility as a complementary biomarker to fGC for identifying adrenal activation. Concentrations of fALD were positively correlated with fGCs in right whales (r = 0.59, P < 0.001), suggesting concurrent secretion of these hormones by the adrenal gland. However, fALD levels were less influenced by concentrations of reproductive steroids in feces, minimizing the potential confounder of assay cross-reactivity in samples with highly skewed hormone ratios. Across different life history states for right whales, fALD concentrations showed similar patterns to those reported for fGC, with higher levels in pregnant females (35.9 + /- 7.6)ng/g) followed by reproductively mature males (9.5 +/- 0.9 ng/g) (P < 0.05), providing further evidence of elevated adrenal activation in these groups of whales. The addition of fALD measurement as a biomarker of adrenal activation may help distinguish between intrinsic and external causes of stress hormone elevations in large whales, as well as other free-living wildlife species, providing a more comprehensive approach for associating adrenal activation with specific natural and anthropogenic stressors.

Campbell-Malone, R., et al. (2008). "Gross and Histologic Evidence of Sharp and Blunt Trauma in North Atlantic Right Whales (Eubalaena glacialis) Killed by Vessels." *Journal of Zoo and Wildlife Medicine* 39(1): 37-55. https://doi.org/10.1638/2006-0057.1

Vessel-whale collision events represented the ultimate cause of death for 21 (52.5%) of the 40 North Atlantic right whales (Eubalaena glacialis) necropsied between 1970 and December 2006. Injuries seen in vessel-struck whales fall into two distinct categories: 1) sharp trauma, often resulting from contact with the propeller, and 2) blunt trauma, presumably resulting from contact with a vessel's hull. This study analyzes four trauma cases that resulted from vessel-whale collisions, which together provide a framework for a more critical understanding of lethal blunt and sharp trauma resulting from vessel collisions with right whales. In case no. 1, contact with a propeller resulted in three deep lacerations. The animal survived acute trauma only to succumb nearly 14 years later when the lesions reopened and became infected. In case no. 2, anecdotal reports linked the laceration of large arteries of the peduncle and histologic evidence of perimortem trauma at a bone fracture site to vessel-whale collision trauma. Case no. 3 had a laceration of the oral rete and a fracture of the rostrum. Both of the areas displayed histologic evidence of perimortem blunt trauma. Finally, in case no. 4, an antemortem mandibular fracture, two additional skull fractures, and widespread hemorrhage were consistent with severe blunt trauma. Evidence from each case, including the timing of trauma relative to the time of death and identifying characteristics of both trauma types, are presented. Before this study, no detailed comparative analysis of trauma pathology that resulted from lethal interactions between vessels and right whales had been conducted. This study demonstrates the importance of detailed gross and histologic examination in determining the significance and timing of traumatic events. This work represents a new paradigm for the differential diagnosis of lethal sharp and blunt trauma in right whales hit by ships and will enhance the present understanding of the impact of anthropogenic mortality on this critically endangered species.

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Corkeron, P., et al. (2017). "A right whale pootree: classification trees of faecal hormones identify reproductive states in North Atlantic right whales (Eubalaena glacialis)." *Conservation Physiology* 5: 9. <a href="https://doi.org/10.1093/conphys/cox006">https://doi.org/10.1093/conphys/cox006</a>

Immunoassay of hormone metabolites extracted from faecal samples of free-ranging large whales can provide biologically relevant information on reproductive state and stress responses. North Atlantic right whales (Eubalaena glacialis Muller 1776) are an ideal model for testing the conservation value of faecal metabolites. Almost all North Atlantic right whales are individually identified, most of the population is sighted each year, and systematic survey effort extends back to 1986. North Atlantic right whales number <500 individuals and are subject to anthropogenic mortality, morbidity and other stressors, and scientific data to inform conservation planning are recognized as important. Here, we describe the use of classification trees as an alternative method of analysing multiple-hormone data sets, building on univariate models that have previously been used to describe hormone profiles of individual North Atlantic right whales of known reproductive state. Our tree correctly classified the age class, sex and reproductive state of 83% of 112 faecal samples from known individual whales. Pregnant females, lactating females and both mature and immature males were classified reliably using our model. Non-reproductive [i.e. 'resting' (not pregnant and not lactating) and immature females proved the most unreliable to distinguish. There were three individual males that, given their age, would traditionally be considered immature but that our tree classed as mature males, possibly calling for a re-evaluation of their reproductive status. Our analysis reiterates the importance of considering the reproductive state of whales when assessing the relationship between cortisol concentrations and stress. Overall, these results confirm findings from previous univariate statistical analyses, but with a more robust multivariate approach that may prove useful for the multiple-analyte data sets that are increasingly used by conservation physiologists.

Fortune, S. M. E., et al. (2012). "Growth and rapid early development of North Atlantic right whales (Eubalaena glacialis)." Journal of Mammalogy 93(5): 1342-1354. https://doi.org/10.1644/11-mamm-a-297.1

Body growth of North Atlantic right whales (Eubalaena glacialis) was described from measurements of known-age live and dead individuals to gain insights into the nutritional needs and life-history strategies of this endangered species. Body lengths from 154 individuals revealed that calves more than doubled in size and attained three-fourths of asymptotic adult size by the time they had weaned at 12 months. Calves gained on average similar to 1.7 cm and similar to 34 kg per day while nursing during this extremely rapid growth phase. Mean predicted lengths and body mass were 4.2 m and 1.1 metric tons (mt) at birth, 10.3 m and 13.5 mt at weaning, and 13.6 m and 29.6 mt when fully grown. Growth of right whales was best described using a 2-phased Gompertz growth model and could not be fit using any of the single continuous growth models commonly used for other mammals. Rapid growth during dependency may minimize the risk of predation and maximize calf survival. Rapid calf growth also may maximize development of the mouth and baleen to optimize foraging efficiency of juveniles at the time of weaning, as well as improve reproductive fitness by reducing the age at which sexual maturity is attained. However, transferring the amount of energy needed to support the rapid postnatal growth of North Atlantic right whales may ultimately affect the intervals between pregnancies (>3 years) of mature females.

Hayes, S. A., et al. (2018). *North Atlantic Right Whales - Evaluating Their Recovery Challenges in 2018*. Dept. of Commerce, National Marine Fisheries Service, Northeast Fisheries Science Center. <a href="https://repository.library.noaa.gov/view/noaa/19086">https://repository.library.noaa.gov/view/noaa/19086</a>

The North Atlantic right whale (Eubalaena glacialis) population has been in decline for 8 years due to increased mortality and sublethal effects from multiple factors. Together these have contributed to a decrease in calving. Shifting ecosystem conditions have also changed North Atlantic right whale behavior and fishing patterns.

Hunt, K. E., et al. (2017). "Multi-year longitudinal profiles of cortisol and corticosterone recovered from baleen of North Atlantic right whales (Eubalaena glacialis)." *General and Comparative Endocrinology* 254: 50-59. https://doi.org/10.1016/j.ygcen.2017.09.009

Research into stress physiology of mysticete whales has been hampered by difficulty in obtaining repeated physiological samples from individuals over time. We investigated whether multi-year longitudinal records of glucocorticoids can be reconstructed from serial sampling along full-length baleen plates (representing ~10 years of baleen growth), using baleen recovered from two female North Atlantic right whales (Eubalaena glacialis) of known reproductive history. Cortisol and corticosterone were quantified with immunoassay of subsamples taken every 4cm (representing ~60d time intervals) along a full-length baleen plate from each female. In both whales, corticosterone was significantly elevated during known pregnancies (inferred from calf sightings and necropsy data) as compared to intercalving intervals; cortisol was significantly elevated during pregnancies in one female but not the other. Within intercalving intervals, corticosterone was significantly elevated during the first year (lactation year) and/or the second year (post-lactation year) as compared to later years of the intercalving interval, while cortisol showed more variable patterns. Cortisol occasionally showed brief high elevations ("spikes") not paralleled by corticosterone, suggesting that the two glucocorticoids might be differentially responsive to certain stressors. Generally, immunoreactive corticosterone was present in higher concentration in baleen than immunoreactive cortisol; corticosterone:cortisol ratio was usually >4 and was highly variable in both individuals. Further investigation of baleen cortisol and corticosterone profiles could prove fruitful for elucidating long-term, multi-year patterns in stress physiology of large whales, determined retrospectively from stranded or archived specimens.

Hunt, K. E., et al. (2006). "Analysis of fecal glucocorticoids in the North Atlantic right whale (Eubalaena glacialis)." *General and Comparative Endocrinology* 148(2): 260-272. https://doi.org/10.1016/j.ygcen.2006.03.012

Very little is known about the endocrinology of the baleen whales. The highly endangered North Atlantic right whale (NARW; Eubalaena glacialis) is a good model species, because most NARW individuals are photo-identified with known histories. We used an 1 251 corticosterone assay, shown to reliably measure cortisol metabolites, to determine glucocorticoid metabolite concentrations in 177 NARW fecal samples collected between 1999-2004 in the Bay of Fundy, Canada. Fecal glucocorticoid metabolite concentrations varied significantly with sex and reproductive category, being highest in pregnant females (mean +/- SE: 238.14 +/- 74.37 ng/g) and mature males (71.6 +/- 11.36), intermediate in lactating females (39.33 +/- 5.82), and lower in nonreproducing females (23.11 + /- 4.25) and immature males (34.33 + /- 5.01) and females (14.0 + /-0.41). One case also suggests that glucocorticoids rise markedly in response to severe entanglement in fishing lines. Whales with fecal glucocorticoid content over 100 ng/g (termed "high-cort" samples) were rare, and included most pregnant females, some mature males, a fatally entangled whale, and several very young animals. Glucocorticoid concentrations were highly correlated with androgen concentrations in males and pregnant females. We analyzed the elution profiles of glucocorticoid and androgen metabolites in 13 samples with high-performance liquid chromatography (HPLC) to determine the extent to which androgen metabolites cross-react with our glucocorticoid assay. Males, pregnant females, non-pregnant females, and "high-cort" whales each had distinctly different immunoreactive HPLC profiles of glucocorticoid and androgen metabolites. A major glucocorticoid metabolite was prominent in all "high-cort" whales including the fatally entangled whale. The major fecal androgen was not testosterone but was instead a more nonpolar steroid (possibly dihydrotestosterone), which may be diagnostic of males. Androgen metabolites showed only minor cross-reactivity to our glucocorticoid assay, having a slight influence on glucocorticoid results in particular individuals. We conclude that fecal glucocorticoid analysis appears to be a useful measure of adrenal activity and reproductive condition for NARW.

Hunt, K. E., et al. (2015). "Conservation Physiology of an Uncatchable Animal: The North Atlantic Right Whale (Eubalaena glacialis)." *Integrative and Comparative Biology* 55(4): 577-586. <a href="https://doi.org/10.1093/icb/icv071">https://doi.org/10.1093/icb/icv071</a>

The North Atlantic right whale, Eubalaena glacialis (NARW), a critically endangered species that has been under intensive study for nearly four decades, provides an excellent case study for applying modern methods of conservation physiology to large whales. By combining long-term sighting histories of known individuals with physiological data from newer techniques (e.g., body condition estimated from photographs; endocrine status derived from fecal samples), physiological state and levels of stress can be estimated despite the lack of any method for nonlethal capture of large whales. Since traditional techniques for validating blood assays cannot be used in large whales, assays of fecal hormones have been validated using information on age, sex, and reproductive state derived from an extensive NARW photo-identification catalog. Using this approach, fecal glucocorticoids have been found to vary dramatically with reproductive state. It is therefore essential that glucocorticoid data be interpreted in conjunction with reproductive data. A case study correlating glucocorticoids with chronic noise is presented as an example. Keys to a successful research program for this uncatchable species have included: consistent population monitoring over decades, data-sharing across institutions, an extensive photo-identification catalog

that documents individual histories, and consistent efforts at noninvasive collection of samples over years. Future research will require flexibility to adjust to changing distributions of populations.

McAloose, D., et al. (2016). "Post-mortem findings in southern right whales Eubalaena australis at Peninsula Valdes, Argentina, 2003-2012." *Diseases of Aquatic Organisms* 119(1): 17. <a href="https://doi.org/10.3354/dao02986">https://doi.org/10.3354/dao02986</a>

Between 2003 and 2012, 605 southern right whales (SRW; Eubalaena australis) were found dead along the shores of Peninsula Valdes (PV), Argentina. These deaths included alarmingly high annual losses between 2007 and 2012, a peak number of deaths (116) in 2012, and a significant number of deaths across years in calves-of-the-year (544 of 605 [89.9%]; average = 60.4 yr(-1)). Postmortem examination and pathogen testing were performed on 212 whales; 208 (98.1%) were calves-f-theyear and 48.0% of these were newborns or neonates. A known or probable cause of death was established in only a small number (6.6%) of cases. These included ship strike in a juvenile and blunt trauma or lacerations (n = 5), pneumonia (n = 4), myocarditis (n = 2), meningitis (n = 1), or myocarditis and meningitis (n = 1) in calves. Ante-mortem gull parasitism was the most common gross finding. It was associated with systemic disease in a single 1-2 mo old calf. Immunohistochemical labeling for canine distemper virus, Toxoplasma gondii and Brucella spp., and PCR for cetacean morbillivirus (CeMV), influenza A, and apicomplexan protozoa were negative on formalin-fixed, paraffin-embedded lung and brain samples from a subset of whales; PCR for Brucella spp. was positive in a newborn/neonate with pneumonia. Skin samples from whales with gull parasitism were PCR negative for CeMV, poxvirus, and papillomavirus. This is the first longterm study to investigate and summarize notable post-mortem findings in the PV SRW population. Consistent, significant findings within or between years to explain the majority of deaths and those in high-mortality years remain to be identified.

Miller, C. A., et al. (2012). "Body shape changes associated with reproductive status, nutritive condition and growth in right whales Eubalaena glacialis and E. australis." *Marine Ecology Progress Series* 459: 135-156. https://doi.org/10.3354/meps09675

Mammalian reproduction is metabolically regulated; therefore, the endangered status and high variability in reproduction of North Atlantic right whales Eubalaena glacialis necessitate accurate assessments at sea of the nutritional condition of living individuals. Aerial photogrammetry was used to measure dorsal body width at multiple locations along the bodies of free-swimming right whales at different stages of the female reproductive cycle (E. glacialis) and during the initial months of lactation (mother and calf Eubalaena australis) to quantify changes in nutritional condition during energetically demanding events. Principal components analyses indicated that body width was most variable at 60% of the body length from the snout. Thoracic, abdominal and caudal body width of E. australis thinned significantly during the initial months of lactation, especially at 60% of body length from the snout, while their calves' widths and width-to-length ratios increased. The body shape of E. glacialis that had been lactating for 8 mo was significantly thinner than non-lactating, non-pregnant E. glacialis. Body shape of E. glacialis measured in the eighth month of lactation was significantly thinner than that of E. australis in the first month, but did not differ from that of E. australis in the third and fourth months. Body width was comparable with diameter calculated from girth of carcasses. These results indicate that mother right whales rely on endogenous nutrient reserves to support the considerable energy expenditure during the

initial months of lactation; therefore, photogrammetric measurements of body width, particularly at 60% of body length from the snout, are an effective way to quantitatively and remotely assess nutritional condition of living right whales.

Moore, M., et al. (2012). "Rope trauma, sedation, disentanglement, and monitoring-tag associated lesions in a terminally entangled North Atlantic right whale (Eubalaena glacialis)." *Marine Mammal Science* 29(2): E98-E113. https://doi.org/10.1111/j.1748-7692.2012.00591.x

A chronically entangled North Atlantic right whale, with consequent emaciation was sedated, disentangled to the extent possible, administered antibiotics, and satellite tag tracked for six subsequent days. It was found dead 11 d after the tag ceased transmission. Chronic constrictive deep rope lacerations and emaciation were found to be the proximate cause of death, which may have ultimately involved shark predation. A broadhead cutter and a spring-loaded knife used for disentanglement were found to induce moderate wounds to the skin and blubber. The telemetry tag, with two barbed shafts partially penetrating the blubber was shed, leaving barbs embedded with localized histological reaction. One of four darts administered shed the barrel, but the needle was found postmortem in the whale with an 80° bend at the blubber-muscle interface. This bend occurred due to epaxial muscle movement relative to the overlying blubber, with resultant necrosis and cavitation of underlying muscle. This suggests that rigid, implanted devices that span the cetacean blubber muscle interface, where the muscle moves relative to the blubber, could have secondary health impacts. Thus we encourage efforts to develop new tag telemetry systems that do not penetrate the subdermal sheath, but still remain attached for many months.

Moore, M. J., et al. (2013). "Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma." *Diseases of Aquatic Organisms* 103(3): 229-+. https://doi.org/10.3354/dao02566

Post-mortem examination of dead and live stranded beach-cast pinnipeds and cetaceans for determination of a cause of death provides valuable information for the management, mitigation and prosecution of unintentional and sometimes malicious human impacts, such as vessel collision, fishing gear entanglement and gunshot. Delayed discovery, inaccessibility, logistics, human safety concerns, and weather make these events challenging. Over the past 3 decades, in response to public concern and federal and state or provincial regulations mandating such investigations to inform mitigation efforts, there has been an increasing effort to objectively and systematically investigate these strandings from a diagnostic and forensic perspective. This Theme Section provides basic investigative methods, and case definitions for each of the more commonly recognized case presentations of human interactions in pinnipeds and cetaceans. Wild animals are often adversely affected by factors such as parasitism, anthropogenic contaminants, biotoxins, subclinical microbial infections and competing habitat uses, such as prey depletion and elevated background and episodic noise. Understanding the potential contribution of these subclinical factors in predisposing or contributing to a particular case of trauma of human origin is hampered, especially where putrefaction is significant and resources as well as expertise are limited. These case criteria descriptions attempt to acknowledge those confounding factors to enable an appreciation of the significance of the observed human-derived trauma in that broader context where possible.

Pettis, H. M., et al. (2017). "Body condition changes arising from natural factors and fishing gear entanglements in North Atlantic right whales Eubalaena glacialis." *Endangered Species Research* 32: 237-249. <a href="https://doi.org/10.3354/esr00800">https://doi.org/10.3354/esr00800</a>

Body condition has been correlated with survival and reproductive success in both terrestrial and marine mammals, including North Atlantic right whales *Eubalaena glacialis*. We used photographs of individually identified right whales to assess visual changes in body condition in reproductive females, adult males, juveniles, and entangled whales. Images from sightings of individual whales were grouped sequentially by habitat region, and each group of images was assigned a body condition score of good, fair, or poor based on the dorsal profile posterior to the blowholes. Temporally consecutive groups of images (n = 1496) of 340 individual whales were compared to investigate the frequency, direction, and minimum timeframe between changes in body condition. Changes in body condition scores of right whales were significantly influenced by group category. Lactating females and severely entangled right whales were more likely to exhibit declining body condition than other groups. Resting females were significantly more likely to improve in condition than other groups but exhibited the longest timeframe for improving condition. Young juveniles were less likely to improve in condition compared to adult males, but remained in compromised condition less frequently than older juveniles and adult males. The shortest timeframes between changing body condition scores were 11 d for declining condition and 12 d for improving condition. This study demonstrates that photographic analysis can detect rapid body condition changes and identifies groups of right whales that are particularly vulnerable to declining condition and delayed recovery from energetically taxing events.

Rolland, R. M., et al. (2005). "Assessing reproductive status of right whales (Eubalaena glacialis) using fecal hormone metabolites." *General and Comparative Endocrinology* 142(3): 308-317. <a href="https://doi.org/10.1016/j.ygcen.2005.02.002">https://doi.org/10.1016/j.ygcen.2005.02.002</a>

Long-term studies of the endangered North Atlantic right whale, Eubalaena glacialis, have revealed declining reproductive parameters over the past two decades, threatening recovery of this small population if current trends continue. Little is known about right whale reproductive physiology, and investigating this reproductive decline has been limited by a lack of non-lethal methods for assessing reproductive status (e.g., sexual maturation, ovarian activity, pregnancy, lactation, and reproductive senescence) in free-swimming whales. This paper describes validation of existing radioimmunoassay techniques to study reproduction in right whales by measuring estrogens, progestins, androgens, and their related metabolites in fecal samples. Over the past decade fecal steroid hormone assays have been used to assess reproductive status and function in a wide range of terrestrial wildlife species, but this is the first application of this methodology in wild cetaceans. Analysis of fecal hormone metabolite levels in combination with life history data from photographically identified whales shows that this non-invasive method can be used to determine gender, detect pregnancy and lactation, and to assess age at sexual maturity in right whales and potentially other endangered whale populations.

Rolland, R. M., et al. (2017). "Fecal glucocorticoids and anthropogenic injury and mortality in North Atlantic right whales Eubalaena glacialis." *Endangered Species Research* 34: 417-429. https://doi.org/10.3354/esr00866

As human impacts on marine ecosystems escalate, there is increasing interest in quantifying sublethal physiological and pathological responses of marine mammals. Glucocorticoid hormones are commonly used to assess stress responses to anthropogenic factors in wildlife. While obtaining blood samples to measure circulating hormones is not currently feasible for free-swimming large whales, immunoassay of fecal glucocorticoid metabolites (fGCs) has been validated for North Atlantic right whales Eubalaena glacialis (NARW). Using a general linear model, we compared fGC concentrations in right whales chronically entangled in fishing gear (n = 6) or live-stranded (n = 1), with right whales quickly killed by vessels (n = 5) and healthy right whales (n = 113) to characterize fGC responses to acute vs. chronic stressors. fGCs in entangled whales (mean +/- SE: 1856.4 + - 1644.9 ng g(-1) and the stranded whale (5740.7 ng g(-1)) were significantly higher than in whales killed by vessels (46.2 +/- 19.2 ng g(-1)) and healthy whales (51.7 +/- 8.7 ng g(-1)). Paired feces and serum collected from the live-stranded right whale provided comparison of fGCs in 2 matrices in a chronically stressed whale. Serum cortisol and corticosterone in this whale (50.0 and 29.0 ng ml(-1), respectively) were much higher than values reported in other cetaceans, in concordance with extremely elevated fGCs. Meaningful patterns in fGC concentration related to acute vs. chronic impacts persisted despite potential for bacterial degradation of hormone meta bolites in dead whales. These results provide biological validation for using fGCs as a biomarker of chronic stress in NARWs.

Rolland, R. M., et al. (2016). "Health of North Atlantic right whales Eubalaena glacialis over three decades: from individual health to demographic and population health trends." *Marine Ecology Progress Series* 542: 265-282. https://doi.org/10.3354/meps11547

Marine mammals are faced with increasing challenges from environmental fluctuation, climate change, and disturbances from human activities. Anthropogenic mortalities have been well documented, but it is difficult to assess the sub-lethal effects of disturbance on the fitness of marine wildlife, and to distinguish these impacts from natural variations in health and reproduction. Here, we used photographic data on body and skin condition, blowhole cyamids, and rake marks, to evaluate the health of North Atlantic right whales Eubalaena glacialis from 1980 to 2008. We applied a hierarchical Bayesian model to these data to estimate the underlying continuous health status of individuals, demographic groups, and the population to characterize health patterns and temporal trends. Visual health scores (scaled from 0 to 100) from 48560 sighting events were used to estimate the health of 622 identified right whales on a monthly basis. Health in most whales fluctuated between 70 and 90, and health scores of <60 were observed in whales in poor condition. Health varied by sex, age-class and reproductive state, with the greatest annual variability occurring in actively reproducing females. Calving females had significantly higher health scores than non-calving females, and a steep deterioration in population health coincided with a dramatic decline in calving from 1998 to 2000. Health in all demographic groups and the population declined over the 3 decades of observations. Given the inevitable data gaps that occur in most marine wildlife research, modeling advances such as the one presented here offer a promising approach to assess the complex interactions between biology, ecology, and sub-lethal anthropogenic disturbance on marine mammals.

Trites, A. W. and D. Pauly (1998). "Estimating mean body masses of marine mammals from maximum body lengths." *Canadian Journal of Zoology* 76(5): 886-896. <a href="https://doi.org/10.1139/cjz-76-5-886">https://doi.org/10.1139/cjz-76-5-886</a>

Generalized survival models were applied to growth curves published for 17 species of cetaceans (5 mysticetes, 12 odontocetes) and 13 species of pinnipeds (1 odobenid, 4 otariids, 8 phocids). The mean mass of all individuals in the population was calculated and plotted against the maximum

body length reported for each species. The data showed strong linearity (on logarithmic scales), with three distinct clusters of points corresponding to the mysticetes (baleen whales), odontocetes (toothed whales), and pinnipeds (seals, sea lions, and walruses). Exceptions to this pattern were the sperm whales, which appeared to be more closely related to the mysticetes than to the odontocetes. Regression equations were applied to the maximum lengths reported for 76 species of marine mammals without published growth curves. Estimates of mean body mass were thus derived for 106 living species of marine mammals.

Tsukrov, I., et al. (2009). "Mechanics of the right whale mandible: Full scale testing and finite element analysis." *Journal of Experimental Marine Biology and Ecology* 374(2): 93-103. <a href="https://doi.org/10.1016/j.jembe.2009.03.012">https://doi.org/10.1016/j.jembe.2009.03.012</a>

In an effort to better understand the mechanics of ship-whale collision and to reduce the associated mortality of the critically endangered North Atlantic right whale, a comprehensive biomechanical study has been conducted by the Woods Hole Oceanographic Institution and the University of New Hampshire. The goal of the study is to develop a numerical modeling tool to predict the forces and stresses during impact and thereby the resulting mortality risk to whales from ship strikes. Based on post-mortem examinations, jaw fracture was chosen as a fatal endpoint for the whales hit by a vessel. In this paper we investigate the overall mechanical behavior of a right whale mandible under transverse loading and develop a finite element analysis model of the bone. The equivalent elastic modulus of the cortical component of right whale mandible is found by comparing full-scale bending tests with the results of numerical modeling. The finite element model of the mandible can be used in conjunction with a vessel-whale collision event model to predict bone fracture for various ship strike scenarios.

#### **Section III: Health Assessments of Other Marine Mammals**

Aguirre, A. A., et al. (1999). "Hawaiian Monk Seal Epidemiology Plan: health assessment and disease status studies." *NOAA technical memorandum NMFS.* U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center. <a href="https://repository.library.noaa.gov/view/noaa/3091">https://repository.library.noaa.gov/view/noaa/3091</a>

The Hawaiian monk seal (Monachus schauinslandi) is one of the most endangered marine mammals in the world. Populations of Hawaiian monk seals have shown a decline in recent years which has placed the species in threat of extinction. Understanding the potential role of disease and toxins is a high priority. Several natural sources of mortality have been identified or suggested (e.g., ciguatera poisoning, starvation, shark predation, trauma/mobbing, and disease), but the relative significance of these factors and their effect on population trends are poorly understood. Efforts to enhance the recovery of the Hawaiian monk seal will require a better understanding of the health and disease status of the wild population. Thus, health and disease impacts on the population merit a cohesive, well-supported effort to mitigate potential effects.

The Epidemiology Plan was developed to prioritize and implement projects regarding health and disease for the Hawaiian monk seal. As a fundamental component of the research and recovery activities conducted by the Marine Mammal Research Program (MMRP), Protected Species Investigation, National Marine Fisheries Service, Honolulu Laboratory, the Epidemiology Plan incorporates specific strategies under the broad heading of health and disease intended to enhance

recovery and prevent further decline of the species. In developing this plan, consideration was given to priorities assigned to specific research tasks outlined in the Recovery Plan for the Hawaiian Monk Seal, Monachus Schauinslandi and the recommendations of the Hawaiian Monk Seal Recovery Team (HMSRT) at its annual meetings. The eventual intent is to develop a long-term plan for addressing various health and disease projects that address management and recovery of the species. For example, disease surveillance, the health and disease aspects of translocation efforts, and the development of contingency plans for unusual mortality and exposure to anthropogenic contaminants, spills, biotoxins, or natural disasters are considered. A component of the plan includes ongoing, prospective health assessment of monk seal subpopulations to monitor temporal changes in health status and to determine the effect on population abundance and reproductive success.

Apprill, A., et al. (2014). "Humpback Whale Populations Share a Core Skin Bacterial Community: Towards a Health Index for Marine Mammals?" *Plos One* 9(3): 17. <a href="https://doi.org/10.1371/journal.pone.0090785">https://doi.org/10.1371/journal.pone.0090785</a>

Microbes are now well regarded for their important role in mammalian health. The microbiology of skin - a unique interface between the host and environment - is a major research focus in human health and skin disorders, but is less explored in other mammals. Here, we report on a crosspopulation study of the skin-associated bacterial community of humpback whales (Megaptera novaeangliae), and examine the potential for a core bacterial community and its variability with host (endogenous) or geographic/environmental (exogenous) specific factors. Skin biopsies or freshly sloughed skin from 56 individuals were sampled from populations in the North Atlantic, North Pacific and South Pacific oceans and bacteria were characterized using 454 pyrosequencing of SSU rRNA genes. Phylogenetic and statistical analyses revealed the ubiquity and abundance of bacteria belonging to the Flavobacteria genus Tenacibaculum and the Gammaproteobacteria genus Psychrobacter across the whale populations. Scanning electron microscopy of skin indicated that microbial cells colonize the skin surface. Despite the ubiquity of Tenacibaculum and Psychrobater spp., the relative composition of the skin-bacterial community differed significantly by geographic area as well as metabolic state of the animals (feeding versus starving during migration and breeding), suggesting that both exogenous and endogenous factors may play a role in influencing the skin-bacteria. Further, characteristics of the skin bacterial community from these freeswimming individuals were assembled and compared to two entangled and three dead individuals, revealing a decrease in the central or core bacterial community members (Tenacibaculum and Psychrobater spp.), as well as the emergence of potential pathogens in the latter cases. This is the first discovery of a cross-population, shared skin bacterial community. This research suggests that the skin bacteria may be connected to humpback health and immunity and could possibly serve as a useful index for health and skin disorder monitoring of threatened and endangered marine mammals.

Bradford, A. L., et al. (2012). "Leaner leviathans: body condition variation in a critically endangered whale population." *Journal of Mammalogy* 93(1): 251-266. https://doi.org/10.1644/11-MAMM-A-091.1

The role of environmental limitation and density-dependent regulation in shaping populations is debated in ecology. Populations at low densities may offer an unobstructed view of basic environmental and physiological interactions that impact individual fitness and thus population productivity. The energy reserves of an organism are reflected in its body condition, a measure

linking individual fitness and the environment. From 1997 to 2007, we monitored the critically endangered western gray whale (Eschrichtius robustus) population on its primary summer feeding ground off the northeastern coast of Sakhalin Island, Russia. This effort resulted in a large data set of photo-identification images from 5,007 sightings of 168 individual whales that we used to visually assess western gray whale body condition. We quantified temporal variation in the resulting 1,539 monthly body condition determinations with respect to observations of reproductive status and sex. Western gray whale body condition varied annually, and we identified years of significantly better (2004) and worse (1999, 2006, and 2007) body condition. This study is the 1st to track the within-season body condition of individual whales. Body condition improved significantly as the summer progressed, although results suggest that not all whales replenish their energy stores by the end of the season. The body condition of lactating females was significantly worse than that of other whales at all times and was most often determined to be compromised. The body condition of their weaning calves exhibited no temporal variation and was consistently good. It is possible lactating females provide an energetic buffer to their offspring at the expense of their own body condition and future reproductive success. Findings from the analysis establish a foundation for quantifying links between western gray whale body condition, demographic parameters, and environmental conditions; and provide a baseline for monitoring individual and population condition of an ecosystem sentinel species in a changing environment. Overall, this study highlights the presence of density-independent environmental and physiological mechanisms that affect the abundance and growth of populations.

The role of environmental limitation and density-dependent regulation in shaping populations is debated in ecology. Populations at low densities may offer an unobstructed view of basic environmental and physiological interactions that impact individual fitness and thus population productivity. The energy reserves of an organism are reflected in its body condition, a measure linking individual fitness and the environment. From 1997 to 2007, we monitored the critically endangered western gray whale (Eschrichtius robustus) population on its primary summer feeding ground off the northeastern coast of Sakhalin Island, Russia. This effort resulted in a large data set of photo-identification images from 5,007 sightings of 168 individual whales that we used to visually assess western gray whale body condition. We quantified temporal variation in the resulting 1,539 monthly body condition determinations with respect to observations of reproductive status and sex. Western gray whale body condition varied annually, and we identified years of significantly better (2004) and worse (1999, 2006, and 2007) body condition. This study is the 1st to track the within-season body condition of individual whales. Body condition improved significantly as the summer progressed, although results suggest that not all whales replenish their energy stores by the end of the season. The body condition of lactating females was significantly worse than that of other whales at all times and was most often determined to be compromised. The body condition of their weaning calves exhibited no temporal variation and was consistently good. It is possible lactating females provide an energetic buffer to their offspring at the expense of their own body condition and future reproductive success. Findings from the analysis establish a foundation for quantifying links between western gray whale body condition, demographic parameters, and environmental conditions; and provide a baseline for monitoring individual and population condition of an ecosystem sentinel species in a changing environment. Overall, this study highlights the presence of density-independent environmental and physiological mechanisms that affect the abundance and growth of populations.

Clegg, I. L. K., et al. (2015). "C-Well: The development of a welfare assessment index for captive bottlenose dolphins (Tursiops truncatus)." *Animal Welfare* 24(3): 267-282. https://doi.org/10.7120/09627286.24.3.267

The field of welfare science and public concern for animal welfare is growing, with the focus broadening from animals on farms to those in zoos and aquaria. Bottlenose dolphins (Tursiops truncatus) are the most common captive cetaceans, and relevant regulatory standards are principally resource-based and regarded as minimum requirements. In this study, the farm animal Welfare Quality (R) assessment was adapted to measure the welfare of bottlenose dolphins, with a similar proportion of animal-based measures (58.3%). The 'C-Well (R)' assessment included eleven criterion and 36 species-specific measures developed in situ at three marine mammal zoological facilities, tested for feasibility and accuracy, and substantiated by published literature on wild and captive dolphins and veterinary and professional expertise. C-Well (R) scores can be calculated for each measure or combined to achieve an overall score, which allows for the comparison of welfare among individuals, demographics, and facilities. This work represents a first step in quantifying and systematically measuring welfare among captive cetaceans and can be used as a model for future development in zoos and aquaria, as well as a means to support benchmarking, industry best practices, and certification.

Gulland, F., et al. (2008). "Health Assessment, Antibiotic Treatment, and Behavioral Responses to Herding Efforts of a Cow-Calf Pair of Humpback Whales (Megaptera novaeangliae) in the Sacramento River Delta, California." *Aquatic Mammals* 34: 182-192. <a href="http://www.cascadiaresearch.org/publications/health-assessment-antibiotic-treatment-and-behavioral-responses-herding-efforts-cow">http://www.cascadiaresearch.org/publications/health-assessment-antibiotic-treatment-and-behavioral-responses-herding-efforts-cow</a>

A mother and female calf humpback whale (Megaptera novaeangliae) pair were observed at an atypical location, 72 nmi inland in the Port of Sacramento, California, on 16 May 2007. Sequencing of mtDNA from a skin biopsy showed the cow to be an E1 haplotype, which is common in the California feeding population. Both animals had lacerations, suggesting sharp trauma from a boat strike. Photographs taken over 11 d showed generalized deterioration of skin condition and necrotic wound edges. Behavioral responses were recorded during attempts to move the animals downriver to the Pacific Ocean. The attempts included playback of alarm tones, humpback and killer whale sounds, banging hollow steel pipes ("Oikami pipes"), spraying water from fire hoses on the water surface, and utilizing tug and power boat engine noise and movement. None of these deterrents resulted in significant, consistent downstream movement by the whales. Antibiotic therapy (ceftiofur) was administered by a dart, representing the first reported antibiotic treatment of free-ranging live whales. After 11 d, the animals swam downstream from fresh water at Rio Vista to brackish water, and their skin condition noticeably improved 24 h later. The animals followed the deep-water channel through the Sacramento Delta and San Francisco Bay, reaching the ocean at least 20 d after first entering the Sacramento River.

Harshaw, L. T., et al. (2016). "Morphometric Body Condition Indices of Wild Florida Manatees (Trichechus manatus latirostris)." *Aquatic Mammals* 42(4): 428-439. https://doi.org/10.1578/am.42.4.2016.428

In many species, body weight (W) increases geometrically with body length (L), so W/L-3 provides a body condition index (BCI) that can be used to evaluate nutritional status once a normal range has been established. No such index has been established for Florida manatees (Trichechus manatus

latirostris). This study was designed to determine a normal range of BCIs of Florida manatees by comparing W in kg with straight total length (SL), curvilinear total length (CL), and umbilical girth (UG) in m for 146 wild manatees measured during winter health assessments at three Florida locations. Small calves to large adults of SL from 1.47 to 3.23 m and W from 77 to 751 kg were compared. BCIs were significantly greater in adult females than in adult males (p < 0.05). W scaled proportionally to L-3 in females but not in males, which were slimmer than females. The logarithms of W and of each linear measurement were regressed to develop amended indices that allow for sex differences. The regression slope for log W against log SL was 2.915 in females and 2.578 in males; W/SL2.915 ranged from 18.9 to 29.6 (mean 23.2) in females and from 24.6 to 37.3 (mean 29.8) in males. Some BCIs were slightly (4%), but significantly (p <= 0.05), higher for females in Crystal River than in Tampa Bay or Indian River, but there was no evidence of geographic variation in condition among males. These normal ranges should help evaluate the nutritional status of both wild and rehabilitating captive manatees.

Hart, L. B., et al. (2013). "Reference ranges for body condition in wild bottlenose dolphins Tursiops truncatus." *Aquatic Biology* 18(1): 63-68. <a href="https://doi.org/10.3354/ab00491">https://doi.org/10.3354/ab00491</a>

Marine mammal body condition, as evaluated by a combination of mass, length, and/or girth measurements, is considered an indicator of nutritional status. We used measurements of total mass, total length, and maximum girth from long-term bottlenose dolphin Tursiops truncatus capture-release research conducted in Sarasota Bay, Florida, USA, (1987 to 2009) to develop 95th percentile reference ranges for 2 body condition models: (1) total mass versus total length and (2) maximum girth versus total length. Nonlinear and linear quantile regression methods were used to estimate the parameters for the reference ranges and develop predictive models to examine body condition among individual dolphins. The flexibility of these models and reliance upon commonly acquired morphometrics allows for broad application among researchers lacking data on mass or age. Ultimately, these reference ranges can be used to evaluate and compare the body condition of individual animals and provide an additional metric for evaluating the general health of coastal populations.

Hart, L. B., et al. (2017). "Rapid Assessment of Bottlenose Dolphin (Tursiops truncatus) Body Condition: There's an App for That." *Aquatic Mammals* 43(6): 635-643. https://doi.org/10.1578/AM.43.6.2017.635

Many recent studies have demonstrated that the health of bottlenose dolphins (Tursiops truncatus) can serve as an important indicator of hazards in the marine environment. A convenient and accurate way to get a "snapshot" of a dolphin's health is to compare biological and physiological measurements to known reference intervals (Ris), which are ranges of values often considered normal or healthy. Measurements that fall above or below RI thresholds are usually considered abnormal and may indicate a health concern. Biological and physiological parameters of individuals sampled during field health assessments are often compared to RIs, but this is usually conducted post fieldwork, following veterinary evaluation and biological sampling, which limits the ability to quickly diagnose problems and immediately perform more telling tests. The objective of this study was to develop a mobile application (app) that allowed instantaneous comparison of bottlenose dolphin morphometrics (i.e., length, mass, and girth) to previously published body condition RIs in situ. Furthermore, for bottlenose dolphins with mass and girth within normal ranges, the mobile app was programmed to compare field measurements to newly derived percentiles (25th, 50th, and 75th). The app was developed using MIT App Inventor 2 (c) software. Functions were validated

using historical and simulated data arid were field tested during a bottlenose dolphin capture-release health assessment to evaluate feasibility for field use and to gain information for feature enhancements. An app that can rapidly evaluate body condition will significantly enhance veterinary evaluations of bottlenose dolphins (in the wild and under human care), as well as enhance epidemiologic studies of population health as coastal environments become increasingly stressed from pollution and other anthropogenic disturbances.

Kershaw, J. L., et al. (2017). "Evaluating morphometric and metabolic markers of body condition in a small cetacean, the harbor porpoise (Phocoena phocoena)." *Ecology and Evolution* 7(10): 3494-3506. <a href="https://doi.org/10.1002/ece3.2891">https://doi.org/10.1002/ece3.2891</a>

Mammalian body condition is an important individual fitness metric as it affects both survival and reproductive success. The ability to accurately measure condition has key implications for predicting individual and population health, and therefore monitoring the population-level effects of changing environments. No consensus currently exists on the best measure to quantitatively estimate body condition in many species, including cetaceans. Here, two measures of body condition were investigated in the harbor porpoise (Phocoena phocoena). First, the most informative morphometric body condition index was identified. The mass/length(2) ratio was the most appropriate morphometric index of 10 indices tested, explaining 50% of the variation in condition in stranded, male porpoises with different causes of death and across age classes (n = 291). Mass/length(2) was then used to evaluate a second measure, blubber cortisol concentration, as a metabolic condition marker. Cortisol is the main glucocorticoid hormone involved in the regulation of lipolysis and overall energy balance in mammals, and concentrations could provide information on physiological state. Blubber cortisol concentrations did not significantly vary around the girth (n = 20), but there was significant vertical stratification through the blubber depth with highest concentrations in the innermost layer. Concentrations in the dorsal, outermost layer were representative of concentrations through the full blubber depth, showed variation by sex and age class, and were negatively correlated with mass/length(2). Using this species as a model for live cetaceans from which standard morphometric measurements cannot be taken, but from which blubber biopsy samples are routinely collected, cortisol concentrations in the dorsal, outermost blubber layer could potentially be used as a biomarker of condition in free-ranging animals.

Wells, R. S., et al. (2004). "Bottlenose Dolphins as Marine Ecosystem Sentinels: Developing a Health Monitoring System." *EcoHealth* 1(3): 246-254. https://doi.org/10.1007/s10393-004-0094-6

Bottlenose dolphins (Tursiops truncatus), as long-lived, long-term residents of bays, sounds, and estuaries, can serve as important sentinels of the health of coastal marine ecosystems. As top-level predators on a wide variety of fishes and squids, they concentrate contaminants through bioaccumulation and integrate broadly across the ecosystem in terms of exposure to environmental impacts. A series of recent large-scale bottlenose dolphin mortality events prompted an effort to develop a proactive approach to evaluating risks by monitoring living dolphin populations rather than waiting for large numbers of carcasses to wash up on the beach. A team of marine mammal veterinarians and biologists worked together to develop an objective, quantitative, replicable means of scoring the health of dolphins, based on comparison of 19 clinically diagnostic blood parameters to normal baseline values. Though the scoring system appears to roughly reflect dolphin health, its general applicability is hampered by interlaboratory variability, a lack of independence between some of the variables, and the possible effects of weighting variables. High score variance seems to indicate that the approach may lack the sensitivity to identify trends over

time at the population level. Potential solutions to this problem include adding or replacing health parameters, incorporating only the most sensitive measures, and supplementing these with additional measures of health, body condition, contaminant loads, or biomarkers of contaminants or their effects that can also be replicated from site to site. Other quantitative approaches are also being explored.

#### **Section IV: Organizations Doing Health Assessments**

"Consortium for Advanced Research on Marine Mammal Health Assessment." from <a href="https://www.carmmha.org/">https://www.carmmha.org/</a>.

The goal of this consortium is to further understand how the toxic effects of oil from the Deepwater Horizon (DWH) disaster impacted cetaceans in the northern Gulf of Mexico. While previous studies have shown that DWH oil exposure led to a variety of adverse physiological effects in dolphins and other marine species-- including increased rates of reproductive failure, lung and cardiac disease, poor body condition, and increased rates of infections-- many questions remain unanswered. Through a combination of thematic projects, field assessments, and integrative modeling, CARMMHA will provide a comprehensive understanding and synthesis of the health impacts of oil-associated chemicals on cetaceans, and a suite of models that will integrate available information collected prior to the spill, immediately following the spill, and over 8 years after the spill, to demonstrate where population-level impacts have occurred and to reassess the current recovery trajectories for Gulf of Mexico cetaceans.

"Pacific Marine Mammal Health Assessments (PMMHA)." from <a href="https://www.nist.gov/programs-projects/pacific-marine-mammal-health-assessments-pmmha">https://www.nist.gov/programs-projects/pacific-marine-mammal-health-assessments-pmmha</a>

Marine mammals have been protected in the United States since 1972 under the Marine Mammal Protection Act (MMPA). The U.S. Pacific Islands and surrounding waters are critical habit for many marine mammal species including the endangered Hawaiian monk seal and northern Pacific humpback whales, which migrate to the waters around Hawaii during the winter to calf and breed. Pacific Marine Mammal Health Assessments (PMMHA) allow monitoring and sample collection from individual living animals in U.S. Pacific Islands marine mammal populations. PMMHA collaborations are diverse including field health evaluations and rehabilitation of endangered Hawaiian monk seals; free swimming cetacean population monitoring through dart biopsy sampling; and following long-term personalized health in bottlenose dolphins under human care.

Consortium, N. A. R. W. (2018). "North Atlantic Right Whale Consortium." Retrieved 19 November 2018, from <a href="https://www.narwc.org/">https://www.narwc.org/</a>.

Started in 1986 as a collaborative data sharing group, the North Atlantic Right Whale Consortium (NARWC) has grown to include more than 200 individuals from various research and conservation organizations, shipping and fishing industries, technical experts, U.S. and Canadian government agencies, and state and provincial authorities, all of whom are dedicated to the conservation and

recovery of the North Atlantic right whale. The Consortium is internationally recognized and has been identified as a model for establishing other species-related consortia.

#### **Section V: Peripheral Materials**

Burek, K. A., et al. (2008). "Effects of climate change on arctic marine mammal health." *Ecological Applications* 18(sp2): S126-S134. <a href="https://doi.org/10.1890/06-0553.1">https://doi.org/10.1890/06-0553.1</a>

The lack of integrated long-term data on health, diseases, and toxicant effects in Arctic marine mammals severely limits our ability to predict the effects of climate change on marine mammal health. The overall health of an individual animal is the result of complex interactions among immune status, body condition, pathogens and their pathogenicity, toxicant exposure, and the various environmental conditions that interact with these factors. Climate change could affect these interactions in several ways. There may be direct effects of loss of the sea ice habitat, elevations of water and air temperature, and increased occurrence of severe weather. Some of the indirect effects of climate change on animal health will likely include alterations in pathogen transmission due to a variety of factors, effects on body condition due to shifts in the prey base/food web, changes in toxicant exposures, and factors associated with increased human habitation in the Arctic (e.g., chemical and pathogen pollution in the runoff due to human and domestic-animal wastes and chemicals and increased ship traffic with the attendant increased risks of ship strike, oil spills, ballast pollution, and possibly acoustic injury). The extent to which climate change will impact marine mammal health will also vary among species, with some species more sensitive to these factors than others. Baseline data on marine mammal health parameters along with matched data on the population and climate change trends are needed to document these changes.

Chen, T. F. L. (2012). *Chromium is a Potential Environmental Health Concern for the North Atlantic Right Whale and Sperm Whale*. Biochemistry and Molecular Biology, University of Maine. Doctor of Philosophy (PhD). <a href="https://digitalcommons.library.umaine.edu/etd/1840/">https://digitalcommons.library.umaine.edu/etd/1840/</a>

Marine metal pollution is a health concern for marine mammals. Marine pollutants that pose health risks to humans were also shown to produce adverse health effects in marine mammals. Although metals can be potent human toxicants they are not commonly studied in great whales. Chromium (Cr) is a metal present in the marine environment but rarely studied in marine mammals. A few studies reported Cr levels in marine mammal tissue but its toxicological effect was not addressed. Cr exists in the marine environment in its trivalent [Cr(III)] and hexavalent [Cr(VI)] forms. Naturally occurring Cr exist mostly as Cr(III), a less potent toxicant than Cr(VI). Cr(VI) is mainly a product of human activities. Studies in humans and animal models show that Cr(VI) cause several adverse effects in multiple biological systems. Cr(VI)-induced health effects include respiratory effects, decreased fertility, depressed immune system and cancer. Underlying these health effects are mechanisms of cellular toxicity, which include cytotoxicity and genotoxicity. The presence of Cr in the marine environment is therefore a potential health concern for marine organisms. Our study investigates Cr (Cr(VI) and Cr(III)) as potential health concerns for marine mammals focusing on great whales. We begin our study with the North Atlantic right whale, a baleen whale with a limited distribution off the eastern coast of North America. Then, we extend our study to the sperm whale, a toothed whale, with a global distribution. We assess and contextualize the toxicity of Cr in these species with two approaches. One approach, direct assessment, involves investigating Cr levels in whale tissue and the cytotoxic and genotoxic effects of Cr in cultured whale cells. The other

approach, contextualization, involves comparing our results in whales with those seen in humans. Our results show that the right whale and sperm whale are exposed to environmental Cr, and that Cr compounds can induce cellular toxicity. Although with some differences, this cellular toxicity is comparable to what is observed in human cells. As a consequence Cr is indeed a potential health concern for the whales and possibly for other marine mammal species as well.

Chen, T. L., et al. (2009). "Cytotoxicity and genotoxicity of hexavalent chromium in human and North Atlantic right whale (Eubalaena glacialis) lung cells." *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 150(4): 487-494. <a href="https://doi.org/10.1016/j.cbpc.2009.07.004">https://doi.org/10.1016/j.cbpc.2009.07.004</a>

Humans and cetaceans are exposed to a wide range of contaminants. In this study, we compared the cytotoxic and genotoxic effects of a metal pollutant, hexavalent chromium [Cr(VI)], which has been shown to cause damage in lung cells from both humans and North Atlantic right whales. Our results show that Cr induces increased cell death and chromosome damage in lung cells from both species with increasing intracellular Cr ion levels. Soluble Cr(VI) induced less of a cytotoxic and genotoxic effect based on administered dose in right whale (Eubalaena glacialis) cells than in human (Homo sapiens) cells. Whereas, particulate Cr(VI) induced a similar cytotoxic effect but less of a genotoxic effect based on administered dose in right whale cells than in human cells. Differences in chromium ion uptake explained soluble chromate-induced cell death but not all of the soluble chromate-induced chromosome damage. Uptake differences of lead ions could explain the differences in particulate chromate-induced toxicity. The data show that both forms of Cr(VI) are less genotoxic to right whale than human lung cells, and that soluble Cr(VI) induces a similar cytotoxic effect in both right whale and human cells, while particulate Cr(VI) is more cytotoxic to right whale lung cells. (C) 2009 Elsevier Inc. All rights reserved.

Chen, T. L., et al. (2009). "Particulate Hexavalent Chromium is Cytotoxic and Genotoxic to the North Atlantic Right Whale (Eubalaena glacialis) Lung and Skin Fibroblasts." *Environmental and Molecular Mutagenesis* 50(5): 387-393. https://doi.org/10.1002/em.20471

Hexavalent chromium compounds are present in the atmosphere and oceans and are established mutagens and carcinogens in human and terrestrial mammals. However, the adverse effects of these toxicants in marine mammals are uncertain. Previously, we reported that North Atlantic right whales, one of the most endangered great whales, have tissue chromium levels that are high, levels that may pose a risk to the whale's health. Furthermore, the study suggested that inhalation may be an important exposure route. Exposure to chromium through inhalation is mainly because of particulate compounds. However, the toxicity of particulate chromium compounds in marine mammal cells is unknown. Accordingly, in this study, we tested the cytotoxic and genotoxic effects of particulate hexavalent chromium in primary cultured lung and skin fibroblasts from the endangered North Atlantic right whale. Cytotoxicity was measured by clonogenic survival assay, and genotoxicity was measured as production of chromosome aberrations. Particulate hexavalent chromium induced cytotoxicity and genotoxicity in a concentration-dependent manner in both right whale lung and skin fibroblasts. Lung fibroblasts were more resistant to chromium cytotoxicity, but presented with more chromosome damage than skin fibroblasts. These data further support the hypothesis that chromium may be a health concern for the endangered North Atlantic right whale.

Hunt, K. E., et al. (2014). "Baleen hormones: a novel tool for retrospective assessment of stress and reproduction in bowhead whales (Balaena mysticetus)." *Conservation Physiology* 2(1): 12. <a href="https://doi.org/10.1093/conphys/cou030">https://doi.org/10.1093/conphys/cou030</a>

Arctic marine mammals are facing increasing levels of many anthropogenic stressors. Novel tools are needed for assessment of stress physiology and potential impacts of these stressors on health, reproduction and survival. We have investigated baleen as a possible novel tissue type for retrospective assessment of stress and reproductive hormones. We found that pulverized baleen powder from bowhead whales (Balaena mysticetus) contained immunoreactive cortisol and progesterone that were detectable with commercially available enzyme immunoassay kits. Both assays passed parallelism and accuracy validations using baleen extracts. We analysed cortisol and progesterone at the base of the baleen plate (most recently grown baleen) from 16 bowhead whales of both sexes. For a subset of 11 whales, we also analysed older baleen from 10, 20 and 30 cm distal to the base of the baleen plate. Immunoreactive cortisol and progesterone were detectable in all baleen samples tested. In base samples, females had significantly higher concentrations of cortisol and progesterone compared with males. Cortisol concentrations in older baleen (10, 20 and 30 cm locations) were significantly lower than at the base and did not exhibit correlations with age-class or sex. Progesterone concentrations were significantly higher in females than in males at all baleen locations tested and were significantly higher in pregnant females than in non-pregnant females. Four of five mature females showed dramatic variation in progesterone concentrations at different locations along the baleen plate that may be indicative of previous pregnancies or luteal phases. In contrast, all males and all immature females had uniformly low progesterone. Baleen hormone analysis is a novel approach that, with further methodological development, may be useful for determining individual longitudinal profiles of reproductive cycles and stress responses.

Martinez-Levasseur, L. M. (2011). "Acute sun damage and photoprotective responses in whales." *Proceedings of the Royal Society B: Biological Sciences* 278(1711): 1581-1586. <a href="http://doi.org/10.1098/rspb.2010.1903">http://doi.org/10.1098/rspb.2010.1903</a>

Rising levels of ultraviolet radiation (UVR) secondary to ozone depletion are an issue of concern for public health. Skin cancers and intraepidermal dysplasia are increasingly observed in individuals that undergo chronic or excessive sun exposure. Such alterations of skin integrity and function are well established for humans and laboratory animals, but remain unexplored for mammalian wildlife. However, effects are unlikely to be negligible, particularly for species such as whales, whose anatomical or life-history traits force them to experience continuous sun exposure. We conducted photographic and histological surveys of three seasonally sympatric whale species to investigate sunburn and photoprotection. We find that lesions commonly associated with acute severe sun damage in humans are widespread and that individuals with fewer melanocytes have more lesions and less apoptotic cells. This suggests that the pathways used to limit and resolve UVR-induced damage in humans are shared by whales and that darker pigmentation is advantageous to them. Furthermore, lesions increased significantly in time, as would be expected under increasing UV irradiance. Apoptosis and melanocyte proliferation mirror this trend, suggesting that whales are capable of quick photoprotective responses. We conclude that the thinning ozone layer may pose a risk to the health of whales and other vulnerable wildlife.

Rosa, C., et al. (2008). "Heavy metal and mineral concentrations and their relationship to histopathological findings in the bowhead whale (Balaena mysticetus)." *Science of The Total Environment* 399(1-3): 165-178. https://doi.org/10.1016/j.scitotenv.2008.01.062

The bowhead whale (Balaena mysticetus) is a species endangered over much of its range that is of great cultural significance and subsistence value to the Inuit of Northern Alaska. This species occupies subarctic and arctic regions presently undergoing significant ecological change and hydrocarbon development. Thus, understanding the health status of the Bering-Chukchi-Beaufort Sea (BCBS) stock of bowhead whales is of importance. In this study, we evaluated the concentrations of six essential and non-essential elements (Zn, tHg, Ag, Se, Cu and Cd) in liver and kidney of bowhead whales (n =64). These tissues were collected from the Inuit subsistence hunt in Barrow, Wainwright and Kaktovik, Alaska between 1983 and 2001. Reference ranges of these elements (including previously reported data from 1983–1997) were developed for this species as part of a health assessment effort, and interpreted using improved aging techniques (aspartic acid racemization and baleen isotopic 13C methods) to evaluate trends over time with increased statistical power. Interactions between element concentrations and age, sex and harvest season were assessed. Age was found to be of highest significance. Sex and harvest season did not affect the concentrations of these elements, with the exception of renal Se levels, which were significantly higher in fall seasons. In addition, histological evaluation of tissues from whales collected between 1998–2001 was performed. Associations between concentrations of Cd in kidney and liver and scored histopathological changes were evaluated. Liver Cd concentration was strongly associated with the degree of lung fibromuscular hyperplasia (P =0.001) and moderately associated with the degree of renal fibrosis (P =0.03). Renal Cd concentration influenced the degree of lung fibromuscular hyperplasia and renal fibrosis (P =0.01). A significant age effect was found for both pulmonary fibromuscular hyperplasia and renal fibrosis, indicating age may be a causative factor. Improvements in aging techniques and the addition of histological indices help clarify the relationships between elements and the influence of life history parameters on concentrations of these elements and potential impacts on health. These data provide essential baseline input useful for monitoring the effects of arctic ecosystem change as it relates to global climate change and industrial development, as well as help inform epidemiological studies examining the public health implications of heavy metals in subsistence foods.

Rosa, C., et al. (2007). "Serum thyroid hormone concentrations and thyroid histomorphology as biomarkers in bowhead whales (Balaena mysticetus)." *Canadian Journal of Zoology* 85(5): 609-618. https://doi.org/10.1139/Z07-035

Serum thyroid hormone (TH) concentrations have been used alone or with other measurements to assess health status or effects of toxicant exposure in marine mammals. Histological sections from thyroid glands of the bowhead whale (Balaena mysticetus L., 1758) were examined in conjunction with serological TH analyses. Serum was assayed for total and free triiodothyronine and total and free thyroxine via radioimmunoassay. Histomorphology of thyroid tissue was assessed by light microscopy and the utilization of an epithelial-follicular index (EFI). Age, sex, or season did not significantly affect serum TH levels. However, TH concentrations in pregnant or lactating females were found to be significantly lower than in the other sex and reproductive groups investigated. The EFI and epithelial height (EH) were greater in spring subadult and adult whales compared with those that were landed in the fall. No correlation was found between serum TH concentrations and serum, blubber, or liver levels of select polychlorinated biphenyl metabolites and organochlorine congeners examined. Low variability in concentrations of the serum THs across age, season, and sex and reproductive groups supports the existence of strong homeostatic mechanisms for maintaining

TH concentrations in these presumably healthy animals. Departures from these ranges may indicate a disturbance in these regulatory mechanisms and may be a useful indication of toxicity or other health disorders.

Vighi, M., et al. (2017). "Bone as a surrogate tissue to monitor metals in baleen whales." *Chemosphere* 171: 81-88. <a href="https://doi.org/10.1016/j.chemosphere.2016.12.036">https://doi.org/10.1016/j.chemosphere.2016.12.036</a>

Metals are massively deposited in the marine environment through direct emissions or atmospheric dry and wet depositions, a process since long enhanced by human activities. Metal contamination in the marine organisms has been increasingly investigated, but most research focuses on few tissues, elements and species considered indicative. Baleen whales have been scarcely studied in this respect. Here we contribute to the fragmented knowledge on this field examining the concentrations of zinc, copper, lead, titanium and strontium in the bone of fin whales (Balaenoptera physalus) from NW Spain and W Iceland. Bone was selected because it is a tissue commonly available in archival historic collections, and it is therefore useful to examine long-term trends in metal pollution. We tested differences between populations and we investigated age- and sex-related accumulation trends, as well as the occurrence of placental transfer. Sr concentrations and Pb accumulation rates with age were significantly higher in individuals from NW Spain than in those from W Iceland. Placental transfer occurred, at different levels, for all metals: as a result fetuses showed significantly higher Cu, Pb and Zn concentrations than adults. After birth, only Zn and Pb concentrations significantly increased with age. Through this study we contributed to fill some gaps in the knowledge regarding metal contamination in marine mammals, and we concluded that bone can be a suitable surrogate tissue to monitor a number of trace elements, provided that dissimilarities in tissue-specific deposition are taken into account when comparing concentrations from different tissues.