An Analysis of Ancient Bowhead Whale *Mangtak* from Gambell Alaska: What can it tell us?

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ABSTRACT

A hunter in Gambell, Alaska discovered a frozen section of bowhead whale epidermis with attached blubber or *mangtak* (in theYupik Eskimo language) from a site within the village about 3.5 m underground, encased in ice. The mangtak was dated using radiocarbon dating techniques at approximately 1,000 year BP but still in relatively "good" condition. Subsequent analyses of a piece of bowhead whale mangtak estimated to be that old is unique in cetacean science. The people of Gambell felt that it confirmed their oral traditional knowledge, which indicates they have hunted bowhead whales since "time immemorial". Scientifically the analysis suggests that: a) the ancient whale is genetically closely related (identical mtDNA haplotype) to whales living within the Bering-Chukchi-Beaufort Seas Stock today (near Alaska); b) bowhead whales 1,000 years ago and today appear to have foraged at the same trophic level, but specific dietary constituents *may* have differed; and c) very low concentrations of mercury in the skin of the ancient (pre-industrial) whale are similar to those found in current day bowhead whale skin.

INTRODUCTION

Inupiat and Siberian Yupik Eskimos have hunted bowhead whales (*Balaena mysticetus*) of the Bering-Chukchi-Beaufort Seas stock (BCBS) continuously for over 2,000 years (Stoker and Krupnik, 1993). Hunting bowhead whales in Alaska remains an important community activity that fulfills important nutritional and cultural needs for several Native (Inuit) communities in Alaska and eastern Chukotka (Russia). Although the species is listed as Endangered under US statute, a highly regulated harvest has been permitted in deference to the significance of bowhead whales in some subsistence communities. Equally important, a long time-series of surveys (1978-2001) demonstrates that the Western Arctic stock of bowhead whales likely numbered at least 10,500 individuals in 2001 and is increasing at approximately 3.4% per year (George *et al.*, 2004).

Despite the encouraging population size and trend, concerns exist about the ability of this highly derived species' ability to cope with natural (e.g., climate and sea ice; food web) and anthropogenically-induced (e.g., industrial, shipping) effects on the Arctic. An integral component in assessments of current and future bowhead status involves the sharing of perspectives acquired through collaborations between Alaska Natives (possessors of traditional knowledge) and a long-term program for scientific research on bowhead whales through the North Slope Borough's Department of Wildlife Management (NSB-DWM). The data and analyses fostered by the NSB-DWM form an important basis for understanding the biology of this obligate polar species and in establishing defendable quotas for subsistence harvests. Collaborations between Natives and scientists have led to unusual insights and opportunities (e.g., George *et al*., 1999).

In August 2005, Douglas Henry of Gambell, Alaska (Saint Lawrence Island), recovered a section of bowhead whale blubber with the epidermis attached (termed *mangtak* in Siberian Yupik and *maktak* in Inupiaq) about 3.5 m beneath the ground (Figure 1). He excavated the mangtak as he recognized that it came from a very old ice cellar and was therefore quite unusual. The blubber was given to J. C. George, who was conducting other research in Gambell during this time, including sampling of bowhead whale bones for DNA to assess structure of the BCBS stock.

The excavated mangtak weighed approximately 40 kg and was about 60 x 40 x 20 cm in dimension. The specimen was taken intact to the DWM laboratories in Barrow, where it was frozen. This unique sample offered the opportunity to provide important insights into bowhead whale biology. Important questions to be addressed were:

1. How old was the mangtak?
2. Was the whale from which the mangtak originated related to whales currently comprising the BCBS or Western Arctic stock of bowheads?
3. Did the diet of the "ancient whale" resemble that of its modern counterparts living in the area?
4. Did the "ancient whale" have detectable and comparable levels of total mercury found in modern Western Arctic bowheads?

METHODS

The specimen was transferred to Barrow and stored in a –20° C freezer. It was then cut into roughly 1 kg sections and distributed to the laboratories for various analyses. In all cases, the results of analyses for the ancient mangtak were compared with results of similar analyses from contemporary bowhead whales taken by subsistence hunters in Barrow and other whaling communities along Alaska's North Slope.

Radio-Carbon Dating

A 4 cm^3 section of the epidermis and blubber was sent to Beta Analytic Inc. (Miami, Florida) for conventional radio-carbon age determination and measured radiocarbon age assessment. Dates are reported as radiocarbon years before present (1950 AD). By international convention, the modern reference standard is 95% of the C^{14} content of the National Institute of Standards and Technology Oxalic Acid and calculated using the Libby C^{14} half-life of 5568 years. Errors represent 1 SD (standard deviation) and are based on combined measurements of the sample, background scatter, and modern reference standards.

Fatty Acid Analysis

A 6 cm^2 x full thickness piece of blubber taken from the interior of the larger sample piece was sent to Mote Marine Laboratory for lipid and fatty acid analyses following the methods described in Wetzel and Reynolds (2004). Briefly, lipids were extracted using dichloromethane and methanol with butylated hydroxytoluene as antioxidant and then derivatized. The resulting lipid mixture was purified using a Florisil™ column to remove sterols and other residual compounds. All samples were analyzed using a Thermo Finnigan DSQ quadrapole GC-MS (Gas Chromatography-Mass Spectrometry).

Stable Isotope Analysis

A 4 cm^2 x full thickness piece of the specimen was shipped to the University of Alaska Fairbanks. Two epidermal sub-samples were freeze-dried for a minimum of 48 hours and ground into a fine powder. For each sample, 0.2–0.4 mg of tissue was weighed into a 4.75 x 4 mm tin capsule, which was folded into a cube. Stable carbon and nitrogen isotope ratios were determined at the

Alaska Stable Isotope Facility at University of Alaska Fairbanks following the procedure described in Dehn et al. (2006a) using a Finnigan MAT Delta^{Plus}XL Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ESC 4010). Enrichment of a particular isotope is reported using the following notation and equation:

$\delta R‰ = ((R_{sample} / R_{standard}) - 1) \times 1000,$

where the differential notation (δR) represents the relative difference between isotopic ratios of the sample and standard gases (i.e., 13C/12C, 15N/14N).

Genetic analysis MtDNA

A 2 cm^3 section of the epidermis was sent to the NOAA Southwest Fisheries Science Laboratory in La Jolla, California, for genetic analyses (Morin et al., 2006). Briefly, DNA was extracted using a PK digestion/silica capture method (SIGMA Cat. # XTR2-IKT, St. Louis, MO) and performed on an X-tractor Gene robotic DNA extraction station (Corbett Robotics Inc., San Francisco, CA). Two separate extractions were performed in isolation of other samples. DNA concentrations were assessed using a 260 lambda reading on a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). DNA was amplified using several primer pairs to obtain 400 base pairs of the 5' end of the mt control region sequence (Table 1). This replication ensured sequence accuracy. Standard protocols were used for PCR product cleaning (Qiagen Qiaquick PCR Purification Kit) and sequencing. Sequencing was done using the same primers as for amplification and Applied Biosystems Big Dye Terminator v3.1 (Applied Biosystems Inc., Foster City, CA). Sequencing products were run on an Applied Biosystems 3100 Genetic analyzer and sequences were aligned using the program Sequencher (v4.1, Gene Codes Corp., Ann Arbor, MI).

Table 1. Primers used in the mtDNA analysis of the ancient blubber.

PRIMER PAIRS – NAME/SEQUENCE	**REFERENCE**	**NUMBER BASES AMPLIFIED/ LOCATION ON 400 BASE SEQUENCE**
TRO- 5'-CCTCCCTAAGACTCAAGGAAG- 3' D- 5'-CCTGAAGTAAGAACAGATG- 3'	Rosel et al. 1994 Developed SWFSC	400/entire
TRO- 5'-CCTCCCTAAGACTCAAGGAAG- 3' A3 - 5'-AATACGRGCTTTAACT- 3'	Developed SWFSC Developed SWFSC	240/beginning
D- 5'-CCTGAAGTAAGAACAGATG- 3' A3r- 5'-GATAAGTTA AAGCTCGTATT- 3'	Rosel et al. 1994 Developed SWFSC	270/end

Total Mercury (THg)

A 10 cm^3 section of the epidermis and blubber was sent to University of Alaska, Fairbanks for analysis of total mercury. Two epidermal sub-samples of the specimen were analysed following the procedure established by Bloom and Crecelius (1983). Tissues were homogenized and digested in a nitric/ sulphuric acid mixture and oxidized using bromine chloride. Mercury in the digest was reduced to Hg^0 with stannous chloride, purged from the liquid digest by argon as a carrier gas, and analysed for total mercury (THg) using a Cetac QuickTrace 7500 cold vapour atomic absorption spectrometer. Mercury analysis was run following standard QA/QC procedures.

RESULTS AND DISCUSSION

Radio-carbon Dating

The age of the sample was estimated using two methods: measured and conventional. The estimated ages were 1,030 ± 70 yr BP and 1,070 ± 70 yr BP, respectively. These age estimates place the specimens in the transition between the late Palaeo-Eskimo and Dorset period (McGhee, 1996). However, age estimates of marine specimen are somewhat difficult to assess due to delayed CO_2 exchange between the atmosphere and the ocean as well as various ocean mixing and dilution effects. This phenomenon is known as reservoir age (Stuiver et al., 1986) and generally a 200 to 400 year correction is applied in a mixed layer system (Stuiver et al., 1998). Dummond and Griffon (2002) found significant variations in carbon-14 age determinations from marine mammal remains in the St. Lawrence Island area and concluded that understanding the reservoir effect in this region was nearly impossible. Nonetheless, even the application of the most conservative correction factors does not change the importance of this historic and unique specimen. To our knowledge, this sample represents the oldest epidermis and blubber sample collected and analyzed for bowhead whales. In addition, the residents of Gambell were enthusiastic as this finding substantiated traditional knowledge that their ancestors have hunted whales for hundreds of years.

Genetic analysis MtDNA

The mtDNA haplotype of the ancient mangtak occurred in about 2.5% of the 400+ modern samples recently analysed as part of the bowhead whale stock structure program (LeDuc et al, In Press). The sequence in the ancient sample is identical to Haplotype 20 from recent bowhead whale samples. No single haplotype is abundant in the sample of modern bowheads; however Haplotype 20 is one of the more common modern haplotypes among living bowheads off Alaska. This suggests that this maternal line has persisted in this population for a minimum of ~1000 years.

Diet

Bowhead whales are baleen whales, adapted to filter-feed on even low-density patches of zooplankton (George et al., 1999). Although a wide variety of prey species have been identified from bowhead stomach contents, their main diet consists of copepods and euphausids (Lowry et al., 2004). In the absence of direct observation of foraging and more classic approaches such as stomach contents and fecal examination, both stable isotope and fatty acid analyses have become powerful tools for dietary reconstructions.

Stable nitrogen isotope ratios become enriched in consumer tissues (compared to the diet) with increasing trophic level, thus allowing for deductions on predator-prey relationships (Kelly, 2000). Carbon isotope signatures on the other hand do not differ substantially between predator and prey. However, stable carbon isotope ratios exhibit spatial gradients and are therefore indicative of feeding habitat (Kelly, 2000). Similarly, many dietary fatty acids are incorporated into consumer tissues without change in chemical structure, transferring the fatty acid signature from prey to predator (Budge et al., 2002).

The fatty acid profile in the ancient blubber was similar in most of the fatty acid constituents, but also had some interesting differences from whale (98B23) harvested near Barrow in 1998 (Figure 2; Wetzel and Reynolds, 2004). The latter individual provides a good basis for comparison because fatty acid analyses were conducted at six separate sites and five depths/site on the body. Since fatty acid composition in bowhead whale blubber varies with sample site (Reynolds *et al.* 2006), having fatty acid data from multiple sites in a modern whale as a basis for comparison is useful since the location on the body from which the ancient mangtak was taken is unknown.

Krahn *et al.* (2001) noted that lipid profiles and concentrations can change as decomposition proceeds resulting in higher proportions of phospholipids, cholesterol, and free fatty acids in decomposed compared to fresh samples. However, even though fatty acids can oxidize over time when exposed to light and/or microbial degradation, these effects may have been minimal due to the storage conditions of the sample and the fact that the subsample that was analysed came form the interior (i.e., unexposed) region of the piece of mangtak. As this sample seemed to be both frozen and out of sunlight over time buried deep within the permafrost, we cautiously speculate that the fatty acid analyses of the ancient blubber may reflect the actual fatty acid constituents when the whale was alive. Approximately 25% of all identified fatty acids were found only in the modern sample, whereas 25% were only identified in the ancient blubber, and about 50% of fatty acids were found in both samples and in similar proportions (Figure 2). The saturated fatty acids accounted for 21% in the ancient blubber and 64% in the modern whale blubber. Monounsaturates were higher in the ancient blubber at 68% compared with 34% in the modern bowhead, and polyunsaturates were 10% and 1.5% respectively. Of the fatty acids that differed between the ancient and modern whale blubber samples, many are likely of dietary in origin, including polyunsaturates such as omega-3 and omega-6 fatty acids, (Reynolds *et al*. 2006). Absent significant changes due to degradation effects from storage, these data suggest that 1,000 years ago this whale may have had a somewhat different foraging behavior or specific prey availability than a modern whale at Barrow. However, locality (Bering vs Beaufort) may also explain some of these differences if some bowheads summered in the Bering/Chukchi Seas as Yankee whaling records suggest (Bockstoce et al, 2005).

Stable nitrogen and carbon isotope ratios of the ancient sample were not significantly different from values published for muscle (P=0.06 and P=0.33 for δ^{15}N and δ^{13}C, respectively) of contemporary bowhead whales (Dehn *et al*., 2006). Again, since this sample was frozen, we suggest that the stable isotope ratios remained fairly stable in the tissues over time. However, Dehn and Follmann (2008) showed that epidermis is enriched in ^{15}N compared to muscle, likely due to differential amino acid composition of the two tissue types and preferential incorporation of ^{15}N in certain amino acids. Nevertheless, epidermal carbon and nitrogen isotope signatures were similar in both present-day bowhead whales and the ancient sample (P=0.07 and P=0.84 for δ^{15}N and δ^{13}C, respectively). Although only a single specimen, this suggests that trophic ecology of bowhead whales has remained stable for a millennium.

To summarize, obviously, conclusions regarding foraging ecology based on a single specimen of unknown sex, length, reproductive condition, and health should be made with caution. For example, stable nitrogen isotope ratios are significantly correlated with length (as a proxy for age) in modern bowhead whales (Dehn et al., 2006; Dehn and Follmann, 2008). Thus, nitrogen isotope signatures are similar in both present-day bowhead whales and the ancient sample only if the ancient sample was taken from an adult whale larger than 10m standard length (Figure 3). Similarly, Reynolds *et al*. (2006) showed that fatty acid profiles in bowhead whale blubber are heterogeneous with regard to location on the body and blubber depth. As noted, storage and decomposition can affect blubber biochemistry and season of death can substantially impact chemical feeding ecology as (modern) bowhead whales typically feed in the isotopically distinct Seas- the Bering, Chukchi and Beaufort Seas (Schell et al., 1989; Lee et al., 2005) during summer.

Total Mercury

Researchers are interested in Mercury levels in very old specimens since there are concerns about temporal increases of Hg over the centuries; i.e., post-industrial revolution, with respect to human and ecosystem health. Bowheads have "low" Hg levels relative to odontocetes but the specimen is still useful in testing for trends in these levels.

Two separate samples (#1 and #2) of epidermis were digested and analysed in duplicate for total Hg. Epidermis sample #1 and #2 contained 5.3 ng/g (ppb) and 3.2 ng/g wet weight (WHAT IS THE SD), respectively. Epidermal mercury in the ancient sample is not significantly different from concentrations in modern whales (P=0.12), but caution is warranted in the interpretation of results. Length (as a proxy for age) is positively correlated to THg in skin. If the ancient specimen came from a juvenile whale (<10m) then THg concentrations are similar in modern and ancient bowhead whales (Figure 4). Compared to adult contemporary whales THg concentrations in the ancient sample appear lower (Figure 4). This is in agreement with studies describing a slight increase in Hg concentrations in hair sampled from mummies in Greenland and Alaska compared to present-day Inuit populations (Toribara and Muhs, 1984; Hansen *et al.*, 1989; Egeland *et al*., 1999). Nevertheless, the THg levels are very low when compared to other cetaceans and are consistent with published values for bowhead whales (Woshner et al. 2001; Dehn et al. 2006). Even though the proportion of organic mercury (MeHg) in skin of Arctic cetaceans is close to 100% (Wagemann *et al*., 1996; Dehn *et al*., 2006), at the low concentrations found in bowhead whales it is likely of little or no toxicological significance.

Gross Anatomy/Histology

Grossly the specimen appears similar to modern whale epidermis and blubber (Figure 1). The skin was black and about 2 cm in thickness. However, the colour of the dermis or blubber was brownish-yellow, different from fresh dermis and was more desiccated than fresh specimens. Considering its age, however, the blubber was remarkably well preserved and retained the distinctive odour associated with marine mammal mangtak.

SUMMARY AND CONCLUSIONS

The accidental discovery and subsequent analyses of a piece of bowhead whale mangtak estimated to be approximately 1,000 years old is unique in cetacean science. The people of Gambell felt that it confirmed their oral traditional knowledge which indicates they have been hunting bowhead whales since “time immemorial”. Scientifically the analysis suggests that: a) the ancient whale was closely related genetically (similar maternal lines) to whales living within the BCBS stock of bowheads whales (i.e., near Alaska) today; b) bowhead whales 1,000 years ago and today forage at the same basic trophic level, but specific dietary constituents and/or composition may differ; c) components of skin (epidermis, blubber, etc.) may present varying signatures of stable isotopes of C and N, and d) mercury analyses indicated that very low concentrations are present which are similar to current day mercury levels for bowhead whale skin.

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Figure 1. Section of the ancient blubber and epidermis (*mangtak*) from an ice cellar at Gambell, Alaska. Note the generally good condition of the specimen. The epidermis is black and still intact; grossly the mangtak is easily recognized and similar to modern mangtak.

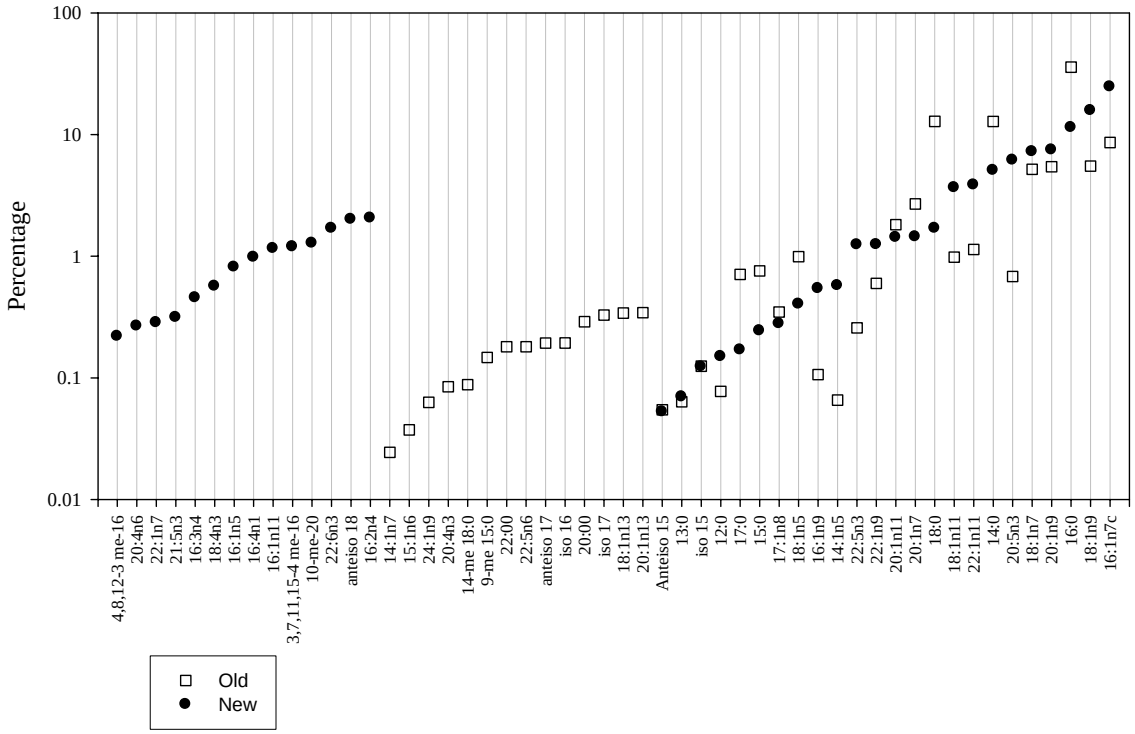

Figure 2. Fatty acid profiles of the ancient blubber. The open squares indicate values for the ancient whale; the black circles are values for a whale harvested at Barrow in 1998 (98B23).

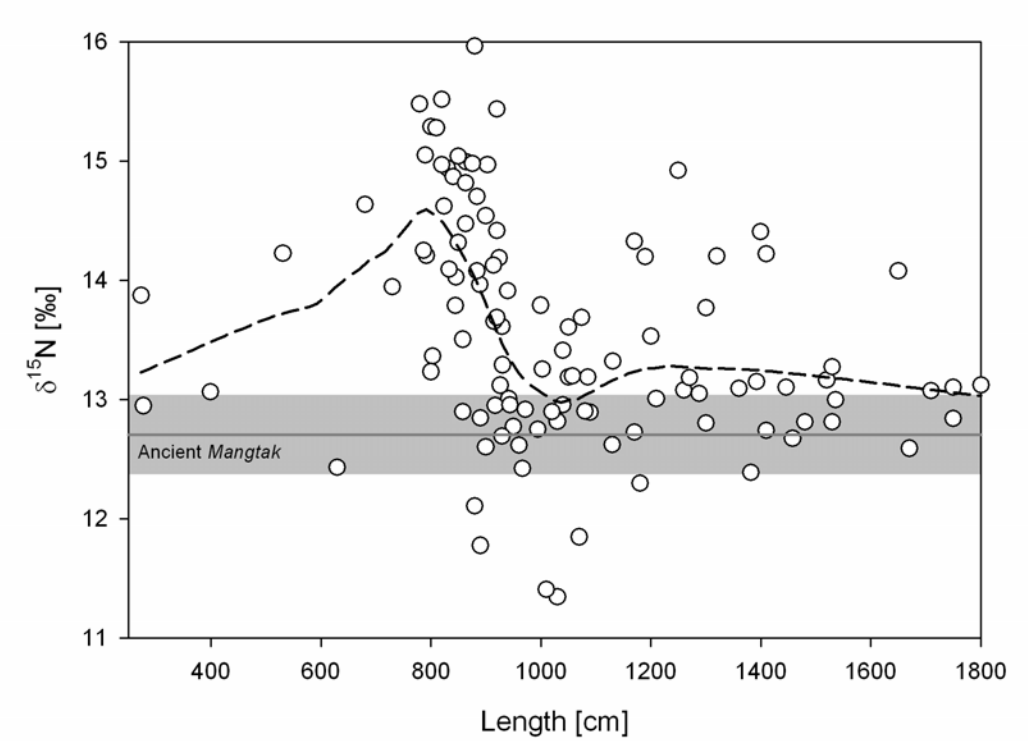

Figure 3. Length [cm] as a proxy for age versus $\delta^{15}N$ in epidermis of modern bowhead whales. The gray line and bar illustrate the mean nitrogen isotope ratio of the ancient epidermis sample ± 1SD (n=4). LOESS non-parametric smoothing was employed to visualize trends.

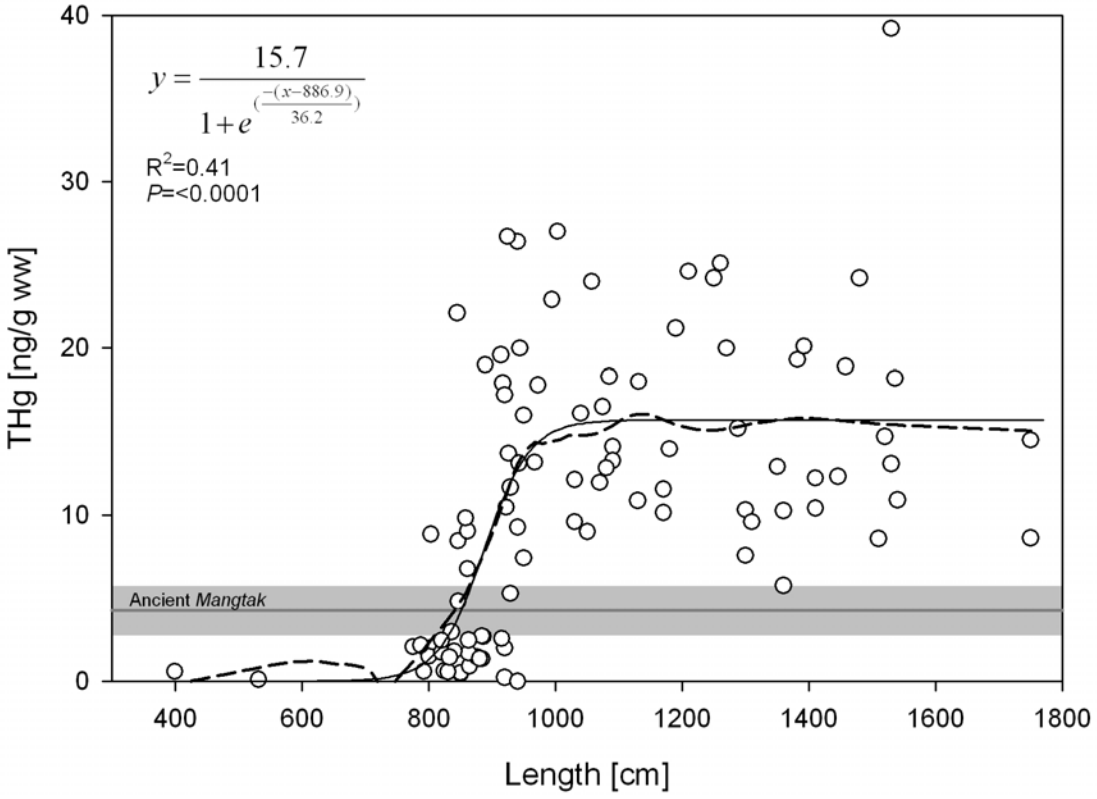

Figure 4. Length [cm] as a proxy for age versus total mercury (THg) in epidermis of modern bowhead whales. The gray line and bar illustrate the mean THg concentration of the ancient epidermis sample ± 1SD (n=2). A sigmoid function was fitted to the data set and LOESS nonparametric smoothing (dashed line) was employed to estimate and compare the regression surface.