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# Application of a Biotechnology Technique for Accurate Identification and Regional Localization of Mammalian Materials in Native American Cultural Heritage

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## EXECUTIVE SUMMARY

Species identification of mammalian materials in historic/ethnographic objects presents a significant challenge to anthropological museums and other collecting institutions due to the limited analytical methods available to conservators, curators, and conservation scientists. Currently, identification of mammalian materials that are processed and worked into cultural objects is mainly through visual and/or tactile examination or based on knowledge of traditional practices and oral history. Although these approaches are valuable, a variety of factors such as material condition and alterations, availability of reference materials, and the expertise of the researcher may limit them. To complement the existing methods, peptide mass fingerprinting[1] (PMF) is lately being applied to the identification of collagen-based mammalian materials[2]. PMF is one of the modern techniques for protein analysis recently introduced into conservation science and requires only micro-samples of material to identify mammalian protein sources to family and, in some cases, species level. Such information has heretofore not been possible, and it adds a higher level of certainty and an important new dimension to the information available to conservators and cultural stakeholders.

This NCPTT-funded project explores the use of PMF to analyze historic and archaeological objects from cultural heritage and to identify material sources contributing to a more complete understanding of the objects and the traditional technologies used to produce them. The project focuses on skin-constructed Alaska Native and Native American objects from the Northwest Coast and High Plains. Through the analysis of 449 samples from 111 objects, the project has not only enhanced documentation and interpretation of specific objects but also confirmed the utility and ease of application of PMF in museum laboratory setting. The project has expanded the library of mammalian references needed for PMF analysis and extended the application of PMF by provisionally identifying markers for new mammalian sources.

The project website and workshop have raised awareness of the PMF method and its wider benefits to cultural community groups, artisans, and conservation researchers. The results and this technical report provide a comprehensive guide to the direct application of PMF to the study of collagen-based objects held in collecting museums and cultural centers.

## 1. Introduction

### 1.1 Project Context, Importance of Materials Identification and Cultural Heritage Objects

The basis for the project's focus on the expanded use of peptide mass fingerprinting (PMF) for the identification of mammalian material sources in cultural objects developed from the collaborative working process at the Peabody Museum with Alutiiq consultants from Kodiak, Alaska. In 2003, a visiting Alutiiq elder identified a rare, early Alutiiq warrior-whaler skin-covered kayak dating to the mid-19$^{th}$ century in the Peabody Museum collection. In 2010, following extensive discussions with curators, conservators, archivists, and Alutiiq consultants, a project focusing on the wider study and conservation of Alaska Native kayaks and kayak-related collections was developed collaboratively and supported in part by a grant from the Save America's Treasures program administered by the Institute of Museum and Library Services. At the beginning of the project in 2011, the importance of

identifying the source of the kayak's skin covering and its sinew stitching was immediately recognized. The Alutiiq consultants wanted to better understand their ancestors' use of materials and their working processes because traditional knowledge is important to the life learning of Alutiiq community members, young and old. DNA analysis of sinew was attempted but produced only inconclusive results. The use of DNA for species identification is often problematic because the DNA molecule can degrade rapidly with age, processing, and other conditions. There have been successes with DNA in some areas, in historical parchments[3], for example, and failures in others, such as archaeological skins.[11] Collins, *et al.*[4] argue that "just as DNA can be considered a barcode of life, collagen has the potential to be the barcode for the communities of the dead." A Harvard conservation scientist, who was involved with the analysis of proteins in artworks, suggested that PMF might be better suited for this kind of sample.

The initial PMF results in 2012 from the warrior-whaler kayak skin were of direct and immediate benefit to the Alutiiq consultants and their understanding of their early cultural heritage. Through oral history, they believed the skin was most likely sea lion; PMF identified the skin specifically and conclusively as seal from the phocini tribe of true or earless seals. It was recognized from this preliminary work that PMF could play an important role in a wider survey of materials from collagen-based objects toward the further understanding of Alutiiq and Alaska Native cultural heritage.

The importance of analytical work, such as described above, is directly aligned with the missions of cultural institutions to enhance collections documentation and make such information widely accessible. Cultural collecting institutions have many different requests for information regarding their collection holdings, but very often information about species identification is unavailable. Identification of mammalian materials used in anthropological objects has principally utilized oral histories and visual and/or tactile examination. These means are important and very valuable, yet can be inaccurate. Based on the initial, positive results with the Alutiiq kayak, PMF was seen as a means of providing accurate identification of materials via a relatively fast and easy technique. To further explore PMF and its practice in a museum conservation laboratory, this NPS National Center for Preservation Technology and Training grant proposal was developed and submitted with support from the Peabody Museum's Alutiiq consultants. The focus of the expanded application of PMF would highlight coastal Alaska Native technologies and, as possible, also examine examples from the Northwest Coast and High Plains areas. Because PMF is based on the comparison of unknowns with authentic reference materials for identification, project activities included gathering reference samples representing expected mammalian populations in the focus areas from several university and museum collections.

The majority of 111 objects analyzed in this study came from the Alutiiq regions of Kodiak Island and coastal Alaska in proximity to Kodiak; from the Aleutian Islands; from Yup'ik regions, including Nunivak Island; and from the Bering Strait, Bristol Bay, Point Barrow and Southeast Alaska. Of this set, 32 were bags/pouches of skin/hide, inner membranes (gut or bladder) or fish skin, and each was sewn with sinew of some type. They were typically complex multi-layered constructions potentially of several different mammalian materials. These small constructed objects were from several different Alaska Native groups, the

majority being from Aleutian/Alutiiq and Yup'ik regions. There were 18 Alaska Native kayak models and two *umiak* boat models made of wood, skin, and inner membrane material and sewn with sinew. Twelve Alaska Native gutskin parkas were of Aleut/Alutiiq manufacture, providing water protection during hunting in the kayaks or, in the case of the more finely embellished and decorated parkas and capes (*kamleikas*), for use in special ceremonies and in official settings. Other clothing items included 10 pairs of boots, caps/hats, mittens, belts, and hair ornaments. Kayak accessories ranged from bladder floats, spears, and harpoons to braided sinew cordage.

To help answer standing questions from curators and Native community representatives, several objects from the Wiyot tribe of northern California, the Northwest Coast, and the High Plains/Upper Missouri River region were folded into this study. Three skin fragments from the Ohio River area, which represented the only pre-historic archaeological specimens in the group of sampled objects, were also included.

All 111 objects in this study were made or collected from the late-18th century to the early-20th century and were from the Peabody Museum's permanent accessioned collections. Location information available from archival documentation ranged from very specific site or island localities to uncertain geographic or cultural regions. Museum records on the objects' materials also varied considerably. For the majority, there was no mention of animal sources for sinew stitching elements and rarely for inner membrane or gut material.

1.2 Identification of Proteinaceous Materials in Cultural Heritage

Proteinaceous materials are an integral part of cultural heritage and are found in many forms fulfilling many functions dating back centuries. Collagen and keratin-based materials are found extensively in objects across all classifications (archaeological, ethnographic, historic, and fine art) such as clothing, tools, religious objects, and decorative art. Detection and identification of proteins in material culture has traditionally relied on FTIR[5] and Raman[6] spectroscopies, amino acid analysis[7], immunological techniques[8], and chemical staining[9]. Recently, new analytical techniques adapted from a variety of fields are being introduced into the work of conservation scientists. For example, modern techniques for protein analysis are being applied to the identification of proteins in artworks[10]. Animal and fish glues are used in grounds for easel paintings, and egg and milk proteins are used as pigment binders. The availability of new methods has provided an exceptional opportunity for conservators and other cultural researchers to obtain information about art and material heritage not previously possible.

PMF[1] is one of the modern techniques for protein analysis recently introduced into conservation science. This methodology requires only micro-samples of material to identify mammalian protein sources to family and, in some cases, species level. Such information has not heretofore been possible.

1.3 PMF Applied to Archaeological and Ethnographic Objects

Species identification of mammalian materials in archaeological and ethnographic objects presents a significant challenge to museums and similar institutions due to the limited analytical methods available to conservators, curators, and conservation scientists. As noted above, examination by tactile and/or visual means (i.e., macroscopic study of the skin's appearance, study of follicle 'grain' patterns on dehaired skins, or via hair

microscopy) have been used for the identification of mammalian materials. Although these approaches can be valuable for the identification of materials where follicle patterns are undisrupted or when hair/fur is present, the results may be debated or inaccurate.[11] Visual and tactile methods rely heavily on the expertise of the examiner and the availability of appropriate references. Visual and tactile observations are ineffectual for the identification of gut and inner membrane materials, where distinctive features are absent. To complement the existing visual/tactile methods, PMF has been adapted to the identification of collagen-based materials[2]. PMF also offers the possibility of determining material origin to the family and possibly species level, adding an important, new dimension to the information available to conservators and cultural stakeholders.

The Peabody Museum's work to identify materials in historic and archaeological ethnographic objects is based on the work of Buckley, *et al.*[12], who demonstrated that PMF could be used to determine mammalian sources of archaeological bones based on differences in amino acid sequences of collagen. The method uses enzymatic digestion of extracted collagen to cleave proteins at specific amino acid sites forming a peptide mixture. Each protein amino acid sequence is unique, thus the mixture of peptides is unique. Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI)[1] is used to analyze the mixture resulting in a mass spectrum containing characteristic marker peptides: a "peptide mass fingerprint." Markers are compared with those from known materials to determine the species from which they were derived. Since few mammalian collagen sequences are known, species identification by PMF requires a reference database. Buckley, *et al* [12] have developed a database for land and sea mammals and use multiple peptide markers as the basis of an identification scheme. In this work, both published markers[2,12, 13, 14] and visual comparison with spectra from reference materials are used for identification.

2. Peptide Mass Fingerprinting Operation Manual

2.1 Methods and Materials/Sampling

Sampling for PMF analysis is micro destructive; that is, a very small physical sample of the material to be analyzed is removed from the object and consumed during analysis. Because of the very small size, samples can usually be obtained without visual impact on the object.

Sampling protocol and considerations:

1. Wear plastic gloves for all sampling operations to avoid contamination.
2. Clean sampling tools between samples with Kim wipe and alcohol to avoid contamination.
3. Place sample in a 600 µL "V-vial" (VWR #89000-010 or equivalent) and verify its presence in the V-vial under a microscope, if necessary. 600 µL vials are preferred for the analysis because they allow the small liquid volumes used for the procedure to vortex high up the tube sides and capture particles into the liquid. All subsequent operations are done in the same V-vial.
4. Record a description of the sample's appearance in the tube. For example, one small fiber, a few small tufts of fibers, a single small-medium size chunk, etc. This information is important for verifying that the sample is in the digestion buffer during subsequent processing.
5. Document the sampling site by assigning a sample number that includes the object accession number and any other relevant information. Photograph the area to be sampled for future location identification.
6. Label the V-vial clearly corresponding to documentation label.
7. Excise or remove samples from an object paying particular attention to avoid contaminating the samples with proteins such as keratins (skin, dust, saliva). Working under a microscope/stereo viewer at 10-30X is very helpful. Use a clean, sharp scalpel, sharp needle or sharp tweezers. For many objects, such as parchment, leather and skin-based objects, samples can conveniently be obtained from a damaged/cracked/hidden area on the object. Tweezers can often be used to pull a fiber or flake free from a damaged area. For parchment, a thin surface flake is usually sufficient. Given the choice, take samples from areas that have had less/least handling, such as turnins on a book covering. Avoid using an artist's brush for sampling as it is easily contaminated. If it is necessary to use a fine brush, for example to transfer staticky particles, clean the brush carefully between samples and inspect it under the microscope for contamination. CAUTION: Static on the tube can cause samples to fly away.
8. Sample size**:** Figures 2 and 3 below illustrate the approximate sample size required for analysis. PMF is extremely sensitive, so very little sample is needed. The sample labeled "small" in figure 2 is more than adequate. Generally if the sample is visible under 30X magnification there is sufficient material for analysis. Larger samples do not interfere with the analysis and may make it easier to verify their presence in the digestion buffer during processing. Larger samples can be sub-sampled, if necessary, and the excess put aside.

Figures 1-6 below show a typical sampling setup (figure 1); sampling tools, site documentation, tube labeling and sample size (figure 2); approximate sample size (figure 3); sampling opportunities at areas of previous damage (figures 4 and 5); and documenting the sampling site (figure 6).

Figure 1. Typical sampling setup with tools, cleaning supplies, and microscope.

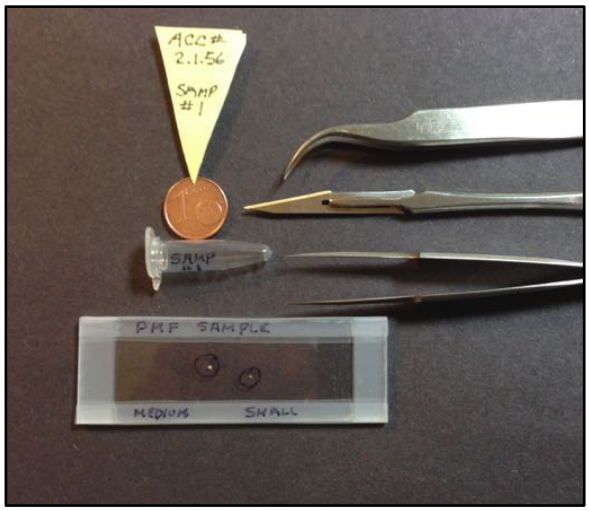


Figure 2. PMF sampling tools, site documentation, sample size, V-vial labeling.


Figure 3. Approximate sample size for PMF.


Figure 4. Sampling locations at area of damage.

Figure 5. Sampling locations at area of damage.

Figure 6. Documenting the sampling site.

## 2.2 Methods and Materials/Solubilization, Reduction, and Alkylation

| | |
|---|---|
| GENERAL CONSIDERATIONS:<br><br>Place samples in a 600 µL "V-Vial." Excise samples from the object/painting, etc., paying particular attention to avoid contaminating the sample with proteins such as keratins (skin, dust, saliva). Use a clean, sharp scalpel or sharp needle. Clean with ethanol, for example, between samples. Avoid using a fine brush as it is easily contaminated. If it is necessary to use a fine brush, for example, to transfer staticky particles, clean the brush carefully between samples and inspect it under the microscope. Transfer samples from microscope slides under a microscope to be sure the sample gets into the digestion tube. | 600 µL tubes are preferred for digestion because they allow the small liquid volumes used in the procedure to vortex high up the tube sides to capture particles into the liquid.<br><br>Many solutions, such as those containing IAA, TCEP and trypsin, can be made ahead and frozen in aliquots until needed. |
| SOLUBILIZATION | NOTES |
| 1. Working in the clean hood, if available, or on a clean bench in a quiet area, add 60 µL 50 mM AMBI (ammonium bicarbonate) to each sample. Vortex and spin (mini centrifuge). The objective is to solubilize the protein sample. | 50 mM AMBI has a pH of approx. 8.3, which is optimal for trypsin activity.<br><br>100 mM AMBI: 79 mg AMBI in 10 mL HPLC grade $H_2O$.<br><br>Dilute to 50 mM and check that the pH is approx. 8.3 with pH paper. Make a fresh solution after 1 week to avoid bacterial growth |
| 2. Heat samples at 80°C for 60 minutes; vortex and spin samples every 15 minutes and recheck that samples are in the buffer liquid. | Check samples under the microscope, if necessary, to make sure everything is in the liquid. If not, flick down solid on walls. The sample must be in the liquid in order for it to react. |
| REDUCTION AND ALKYLATION | NOTES |
| 3. Cool samples to RT.<br><br>Add 3 µL 20 mM TCEP (tris (2-carboxyethyl) phosphine hydrochloride) in 25 mM AMBI. Heat to 37°C for 20 min. | TCEP reduces disulfide bonds in proteins to thiols aiding in solubilization and tryptic digestion.<br><br>20 mM TCEP in 25 mM AMBI: 57.3 mg TCEP in 10 mL 25 mM AMBI. Freeze 100-200 µL aliquots until needed. |
| Add 3 µL 40 mM IAA (iodoacetamide) in 25 mM AMBI. Allow to react 30 min., RT, dark. | IAA end caps the thiols formed in the previous step, and prevents them from recombining.<br><br>40 mM IAA in 25 mM AMBI: 74 mg IAA in 10 mL 25 mM AMBI. Freeze 100-200 µL aliquots until needed. Protect from light. |

Table 1. Procedure for solubilization, reduction, and alkylation of protein samples.

2.3 Methods and Materials/Digestion and Sample Preparation for MALDI

| DIGESTION | NOTES |
|---|---|
| 5. Add 8 µL trypsin (0.02 ug/µL in 50 mM AMBI). | Trypsin digests protein into predictable peptides by cutting the protein on the C-terminal side of lysine (K) and argentine (R).<br><br>Trypsin 0.02 µg/µL 50 mM AMBI: Add 1 mL 50 mM AMBI to a vial containing 20 ug lyophilized trypsin. Freeze 100 µL aliquots until needed. |
| 6. Digest at 37°C at least 2 hr. but preferably over night. | Digestion may be essentially complete in as little as 2 hrs. For convenience, digests are usually left overnight. |
| SAMPLE PREPARATION FOR MALDI | NOTES |
| 7. Add 1 uL formic acid or 1 uL 0.1% trifluoroacetic acid (TFA). Vortex and spin solids to bottom of tube. Vacuum centrifuge as necessary to reduce volume. | Addition of acid stops the trypsin digestion reaction by lowering the pH. If samples have been digested overnight this step may be omitted. |
| 8. Prepare Matrix:<br><br>Saturated CHCA (α-cyano-4-hydroxycinnamic acid) in 40% (v/v) ACN (Acetonitrile) 0.1% (v/v) TFA | Add a small amount of CHCA to a glass vial, add 40% ACN 0.1% TFA, vortex intermittently for 5 minutes and allow undissolved CHCA to settle. If undissolved CHCA is present, the solution is saturated. |
| 9. Prepare samples:<br><br>Combine 20 µL matrix and 2-3 µL sample in a new 600 µL tube. Vortex and spin. Spot ~1.5 µL on the MALDI plate. Allow to air dry. | Do not allow pipette tip to touch the MALDI plate as this interferes with proper matrix crystallization. Check dried spots under the microscope to observe proper crystallization. |
| 10. Prepare peptide standard: 3 µL standard and 20 µL matrix. Spot ~1 µL on the MALDI plate. Allow to air dry. | Do not allow pipette tip to touch the MALDI plate. |

Table 2. Procedure for digestion and sample preparation for MALDI.

Figure 7 shows a typical bench top setup for sample processing.

Peptide standard mixtures are available from instrument manufacturers and should include the range of 700 to 3500 Da for PMF analysis.

When samples are dried on the sample plate, they are stable for at least several weeks if kept from light and can be transported to available equipment when convenient.

The equipment and reagents described in Section 2.8 Methods and Materials/PMF Consumables and Equipment are typical for PMF analysis, and substitutions can be made. If there is any doubt about specific equipment, reagents, or procedures, a positive control such as lab gelatin can be used to validate/troubleshoot the method. Figure 8 is a reference spectrum for lab gelatin (cattle) for comparison. Cattle markers as well as other intense ions are labeled.

MALDI analysis can be conducted on any instrument capable of covering a mass range of 800-3300 Da with resolution of at least 10, 000 m/Δm at ~2500 Da.

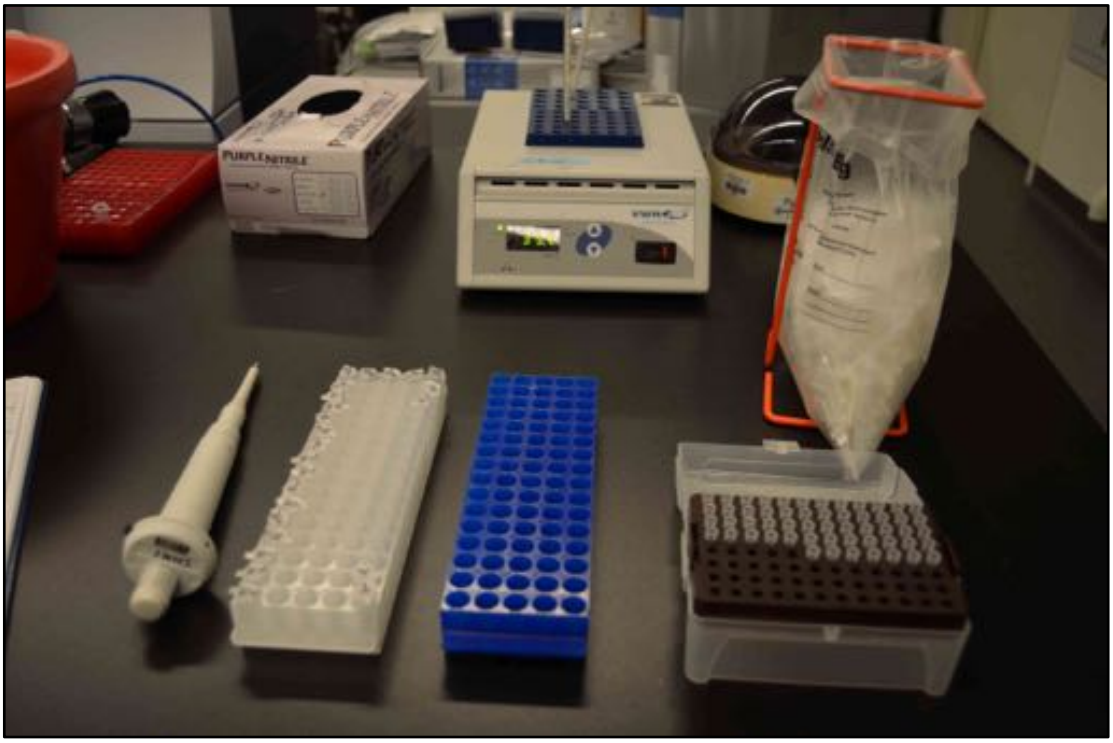

Figure 7. Typical bench top setup for sample preparation. © 2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology (digital file# 99250036).

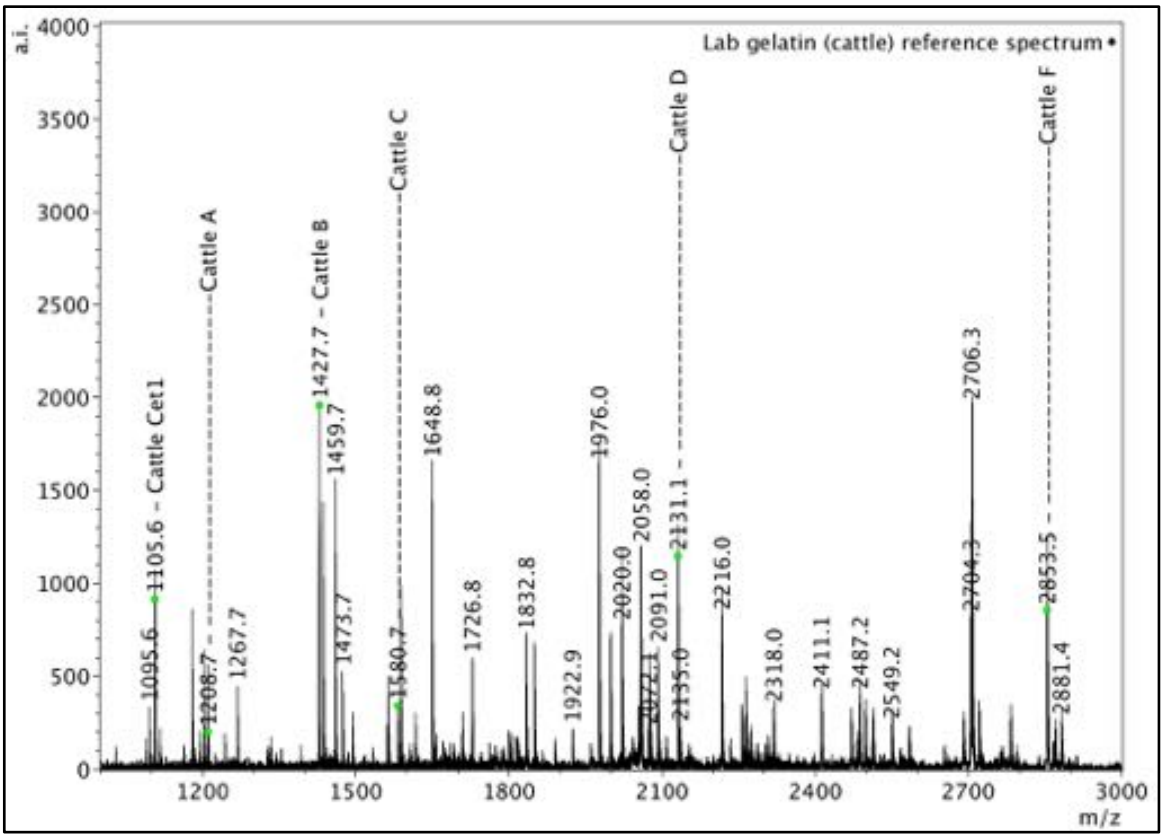


Figure 8. PMF from lab gelatin (cattle) reference.

2.4 Methods and Materials/ZipTip® Fractionation

Occasionally it may be necessary to fractionate the final digestion mixture and obtain MALDI spectra of each fraction to clarify/intensify certain marker ions, particularly the A, D and/or G markers. If marker ions are clearly present at a S/N > 3, fractionation is unnecessary.

2.4.1 ZipTip Fractionation Procedure (10/50%)[12]:

1. Equilibrate the ZipTip with 2-3 X 10 µL 80% ACN, 0.1% TFA
2. Equilibrate the ZipTip with 2-3 X 10 µL 0.1 % TFA
3. Load 1-10 µL sample (depending on the amount of material in the sample. Do not overload.)
4. Wash with 2-3 X 10 µL 0.1% TFA
5. Elute with 10 µL 10% ACN, 0.1% TFA into a clean tube (10% fraction)
6. Elute with 10 µL 50% ACN, 0.1% TFA into a clean tube (50% fraction)
7. Add 10-20 µL CHCA matrix (see above, digestion) to 10% and 50% fractions
8. Spot ~1 µL of each fraction onto the MALDI plate

Figure 9 compares the original sample and the 50% fraction from the same sample to confirm the presence of the G marker at 3059 Da.

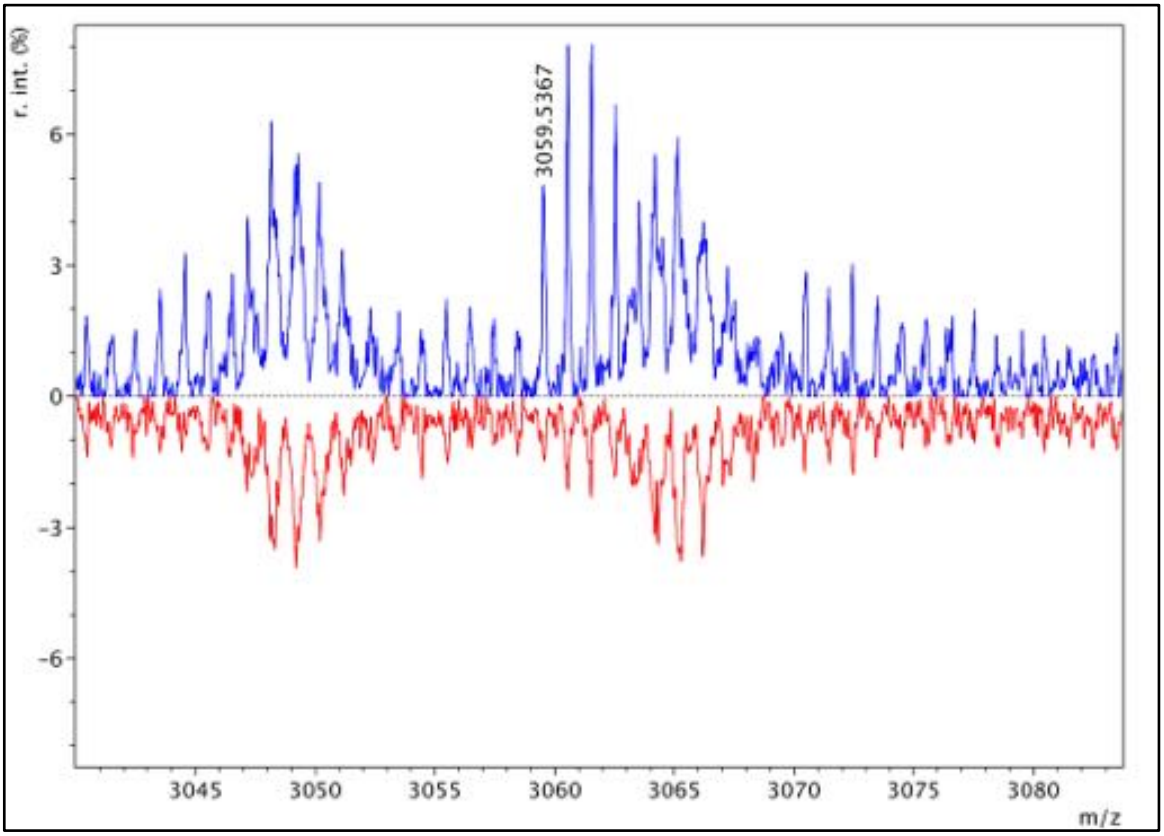


Figure 9. Original sample (red, lower) and 50% fraction (blue, upper) clarifying the presence of a G marker at 3059 Da.

In some instances it may be necessary to clarify the G marker to distinguish between goat and sheep. In this case the 22/26/32 fractionation method is used.

2.4.2 ZipTip Fractionation Procedure (22/26/32%)[15]:

1. Equilibrate the ZipTip with 2-3 X 10 µL 80% ACN, 0.1% TFA
2. Equilibrate the ZipTip with 2-3 X 10 µL 5% ACN, 0.1 % TFA
3. Load 1-10 µL sample (depending on the amount of material in the sample. Do not overload.)
4. Wash with 2-3 X 10 µL 5% ACN, 0.1% TFA
5. Elute with 10 µL 22% ACN, 0.1% TFA into a clean tube (22% fraction)
6. Elute with 10 µL 26% ACN, 0.1% TFA into a clean tube (26% fraction)
7. Elute with 10 µL 32% ACN, 0.1% TFA into a clean tube (32% fraction)
8. Add 10-20 µL CHCA matrix (see above, digestion) to each fraction
9. Spot ~1 µL of each fraction onto the MALDI plate

The G marker will be visible in the either the 26% fraction or the 32% fraction, or both, with enhanced intensity and reduced background.

Figure 10 compares the original sample with the 26% fraction for the same sample clarifying the presence of the G marker at 3033 Da.

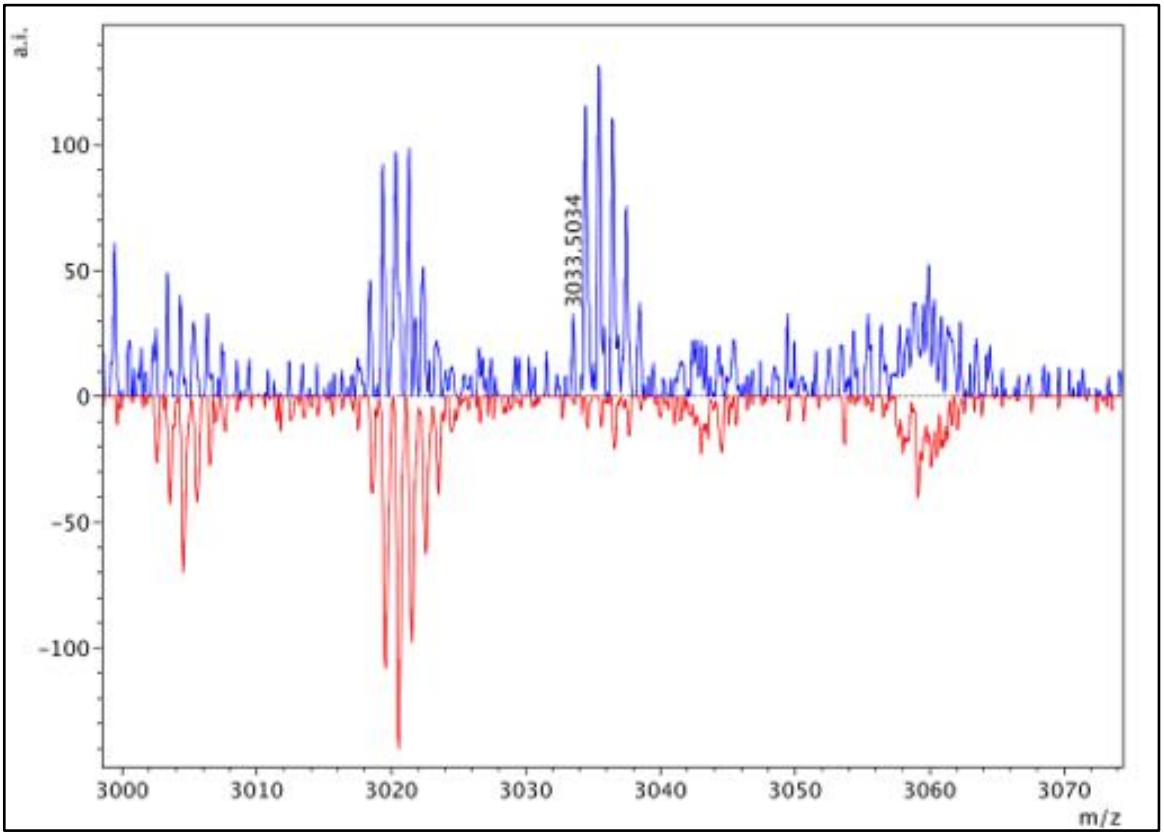


Figure 10. Original sample (red, lower) and 26% fraction (blue, upper) clarifying the presence of a G marker at 3033 Da.

2.5 Methods and Materials/Data Analysis with mMass

Following the extraction/digestion procedure and MALDI analysis, the MALDI spectra are analyzed using mMass, a freeware program available at: www.mmass.org. This program is used to search PMF spectra for markers or, in cases where markers have not yet been determined, to compare PMF's visually with reference spectra. mMass can be used to manipulate data, annotate spectra, apply calibration, and export spectra as jpg, png, or tiff image files. From within mMass, mass lists from unknowns can be sent to MASCOT[16] for database searching. Database searching usually does not provide a specific identification because of the lack of public sequence data but may be able to help classify an unknown as fish or bird, for example.

The mMass package contains an excellent User's Guide covering every aspect of the program's capabilities. The following is a "quick start" guide for analyzing PMF MALDI data.

Processing PMF spectra with mMass

1. Download the mMass software from www.mmass.org. In the User's Guide, the "Spectrum Manipulations" section is especially helpful for understanding how to zoom in and move the spectrum on the screen (User's Guide p.26).
2. The input files for mMass are .txt files, which can be exported from the MALDI instrumentation. These are simple x, y files with pairs of mass and intensity data. Load .txt files into mMass by either dragging them into the program or opening them via **File → Open**.
3. Smooth the spectra: **Processing → Smooth Spectrum**. Parameters should be chosen so that they reduce baseline noise and smooth mass peaks without significantly changing peak intensity or location.
4. Apply baseline correction: **Processing → Baseline Correction**. Parameters should correct any baseline irregularities without producing any artifacts, such as baseline dips below zero intensity.
5. Apply peak picking: **Processing → Peak Picking**. Parameters should include "deisotoping" so that only the monoisotopic mass is labeled.
6. Save the processed spectrum as an .msd (Mass Spectrum Document) file: **File → Save.** Saved files reopened in mMass will contain all the processing results. The original .txt file will remain unchanged.

## 2.6 Methods and Materials/Alaskan Mammals

Based on several references,[17, 18, 19] the Alaskan mammals used as source material in Alaska Native objects, clothing, and accessories are shown in Table 3. All of these materials can be identified at least to family level with either PMF markers or new reference materials added in this work.

| Sea mammals | Land mammals |
|---|---|
| Beluga whale (Delphinapterus leucas) # | Arctic fox (Alopex lagopus) # |
| Blue whale (Balaenoptera musculus) # | Arctic ground squirrel (Sepmophilus parryii) @ |
| Dall porpoise (Phocoenoides dalli) # | Arctic hare (Lepus arcticus) # |
| Fin whale (Balaenoptera physalus) # | Badger (Taxidea taxus taxus) # |
| Gray whale (Eschrichtius robustus) # | Beaver (Castor canaadensis) @ |
| Grey seal (Halichoerus grypus) # | Black bear (Ursusu americanus) # |
| Harbor porpoise (Phocoena phocoena) # | Brown grizzly bear (Ursus arctos) # |
| Harbor seal (Phoca vitulina) # | Caribou (Rangifer tarandus) @ |
| Harp seal (Pagophilus groenlandicus) # | Dall sheep (Ovis dalli) # |
| Humpback whale (M. novaeangliae)# | Dog / wolf (Canis) # |
| Minke whale (balaenoptera acutorostrata) # | Marmot (Marmota caligata oxytona) @ |
| Northern fur seal (Callorhinus ursinus) # | Mink (Neovison vison) @ |
| Northern right whale (E. glacialis) # | Moose (Alces alces) @ |
| Orca (Orcinus orca) # | Mountain goat (Oreamnos americanus) # |
| Bearded seal (Erignathus barbatus) # | Musk ox (Ovibos moschatus) # |
| Pacific walrus (Odobenus rosmarus) # | Muskrat (Ondatra zibethicus) @ |
| Pacific whitesided dolphin(L. obliquidens)# | Red fox (Vulpes vulpes) # |
| Ribbon seal (Phoca fasciata) # | Red Squirrel (Tamiasciurus hudsonicus @ |
| Ringed seal (Phoca hispida) # | Roosevelt elk ( Cervus canadensis) # |
| River otter (Lontra canadensis) @ | Short-tailed weasel (Mustela erminea) @ |
| Sea otter (Enhydra lutris) @ | Sitka black tailed deer (O. hemiorius sitkensis) # |
| Steller sea lion (Eumetopias jubatus) | Snowshoe hare (Lepus americanus) # |
| | Weasel (Mustela nivalis) @ |

Table 3. Sea and land mammals traditionally used for Alaska Native objects. (#) Included in published markers (table 4). (@) Included in potential new marker (table 4).

## 2.7 Methods and Materials/Table of Markers

Table 4 lists markers used to identify land and sea mammals in this project. Markers shown in black are consolidated from several publications;[2,12,13,14] those shown in red are provisional markers based on the analysis of new reference materials. The new marker ions are tentatively assigned based on comparison with masses of known markers. Conclusive validation requires LCMSMS (liquid chromatography/tandem mass spectrometry) analysis to verify peptide sequences, which is not part of this work. It should be noted that all material identifications reported here are based on the markers in table 4 or by comparison with new reference PMF's, table 6. All mammalian material sources expected in the project's focus area (table 3) are included in the database and reference spectra. However, the possibility that other mammalian sources with the same or very similar markers might be present in some objects must also be considered.

Cet1 and Cet2 markers are used to differentiate cetaceans from other mammals. (P0) Ref, (P1) Ref, (P2) Ref and (Set3) Ref are additions to the original scheme of markers[12] and are useful for separating cetaceans [(P1) and (P2)] or for differentiating among various land and sea mammals [(P0) and (Set3)]. Most material identifications reported here are made

using the original A, B, C, D, F and G markers[12]. If present, other markers are used as additional confirmation of identity. In certain cases, visual comparison of PMF spectra from an object sample and a reference was used to narrow the possibilities among very similar mammals. See 3.3.1 Child's boots, for example, where mustelidae family (ermine, mink, or weasel) and muskrat identifications were made in this manner.

As shown in the first column, table 4, some mammals cannot be identified beyond the family level, for example Northern fur seal and Steller sea lion (otariidae, eared seals), wolf and dog (canidae), and black, brown and polar bears (ursidae). Discovering additional markers in PMF spectra that might allow in-family identification is a consideration for future work.

| | Cet1 | (A) | (B) | (C) | Cet2 | (P0) REF | (D) | (P1) REF | (P2) REF | (Set3) REF | (F) | (G) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Walrus | 1105 | 1221 | 1453 | 1566 | 1652 | | 2121 | 2246 | 2342 | 2731 | 2853 | 3003 |
| Northern fur seal/ Steller sea lion | 1105 | 1221 | 1453 | 1566 | 1652 | | 2121 | 2216 | 2342 | 2757 | 2853 | 2957 |
| Bearded seal | 1121 | 1221 | 1453 | 1566 | 1652 | | 2171 | 2216 | 2332 | 2755 | 2853 | 2957 |
| Ringed seal | 1105 | 1221 | 1453 | 1566 | 1652 | | 2171 | 2232 | 2346 | 2743 | 2869 | 2957 |
| Phocini seal: ribbon, spotted, grey, harbor, harp | 1105 | 1221 | 1453 | 1566 | 1652 | | 2171 | 2216 | 2346 | 2743 | 2869 | 2957 |
| Hooded seal | 1105 | 1221 | 1453 | 1566 | 1652 | | 2171 | 2216 | | | 2853 | 2957 |
| Cattle / Bison | 1105 | 1208 | 1427 | 1580 | | | 2131 | 2199 | | 2767 | 2853 | 3033 |
| Sheep/Pronghorn | 1105 | 1196 | 1427 | 1580 | | | 2131 | 2199 | | 2767 | 2883 | 3033 |
| Goat | 1105 | 1196 | 1427 | 1580 | | | 2131 | 2199 | | 2767 | 2883 | 3093 |
| Musk Ox | 1105 | 1208 | 1427 | 1580 | | | 2131 | 2199 | 2348 | 2769 | 2883 | 3033 |
| Elk/red deer/fallow deer | 1105 | 1196 | 1427 | 1550 | | | 2131 | 2199 | | 2767 | 2883 | 3033 |
| Caribou/reindeer | 1105 | 1166 | 1427 | 1580 | | | 2131 | 2199 | | 2767 | 2883 | 3093 |
| Roe deer | 1105 | 1196 | 1427 | 1550 | | | 2131 | 2199 | | 2769 | 2883 | 3059 |
| North American deer: mule, Sitka, whitetail | 1105 | 1196 | 1427 | 1580 | | | 2131 | 2199 | | 2767 | 2883 | 3059 |
| Horse | 1105 | 1198 | 1427 | 1550 | 1682 | | 2145 | | | | 2883 | 2999 |
| Dolphin: common, bottlenose, white-beaked, euphrosyne | 1079 | 1205 | 1453 | 1566 | 1638 | | 2119 | 2225 | | 2767 | 2883 | 3023 |
| Risso's Dolphin /pilot whale / false killer whale | 1063 | 1205 | 1453 | 1566 | 1638 | | 2119 | 2225 | | 2767 | 2883 | 3023 |
| Orca / White-sided dolphin | 1079 | 1205 | 1453 | 1566 | 1652 | | 2119 | 2225 | | 2767 | 2883 | 3023 |
| Porpoise | 1079 | 1205 | 1453 | 1550 | 1652 | | 2119 | 2225 | | 2767 | 2883 | 3023 |
| Narwhal | 1079 | 1205 | 1443 | 1550 | 1652 | | 2089 | 2225 | | 2777 | 2883 | 3051 |
| Beluga whale | 1079 | 1205 | 1443 | 1550 | 1652 | | 2121 | 2225 | | 2777 | 2883 | 3051 |
| Sperm whale | 1079 | 1205 | 1453 | 1550 | 1652 | | 2133 | 2225 | | 2747 | 2883 | 3039 |
| Bottlenose / Sowerby's whale | 1063 | 1205 | 1441 | 1550 | 1638 | | 2091 | | | | 2883 | 3023 |
| Minke whale | 1079 | 1205 | 1441 | 1566 | 1652 | | 2135 | 2225 | | 2757 | 2883 | 3023 |
| Fin Whale | 1079 | 1205 | 1453 | 1566 | 1652 | | 2135 | 2225 | | 2757 | 2883 | 3023 |
| Humpback whale | 1079 | 1205 | 1453 | 1566 | 1652 | | 2135 | 2225 | | 2777 | 2869 | 3023 |
| Blue whale | 1079 | 1205 | 1453 | 1550 | 1652 | | 2105 | | | 2757 | 2883 | 3023 |
| Gray whale | 1079 | 1205 | 1453 | 1566 | 1652 | | 2135 | 2225 | | | 2899 | 3023 |
| Sei whale | 1079 | 1205 | 1441 | 1550 | 1652 | | 2135 | | | | 2883 | 3023 |
| Right whale | 1079 | 1205 | 1453 | 1566 | 1682 | | 2135 | 2225 | | 2789 | 2883 | 3023 |
| Elephant/Mastodon | 1105 | 1251 | 1453 | 1579 | | 1933 | 2115 | 2199/2216 | 2348 | 2743 | 2853 | 2999/3015 |
| Black rhino | 1105 | 1184/1198 | 1453 | 1550 | | | 2145 | | | | 2869 | 2999 |
| Fox: red, Arctic | 1105 | 1210/1226 | 1437 | 1566 | | | 2131 | | | | 2853 | 2999 |
| Gray fox | 1105 | 1208/1224 | 1453 | 1566 | | | 2131 | 2216 | 2342/2348 | | 2853 | 2899 |
| Cat | 1105 | 1207/1223 | 1453 | 1566 | | | 2163 | | | | 2853 | 2999 |
| Pig | 1105 | 1180/1196 | 1453 | 1550 | | | 2131 | | | | 2883 | 3033 |
| Rabbit | 1105 | 1221/1235 | 1453 | 1550? | | | 2129 | | | | 2883 | 2957 |
| Rat | 1105 | 1187/1203 | 1453 | 1566 | | | 2143 | | | | 2883 | 3003 |
| Mouse | 1105 | 1178/1194 | 1453 | 1566 | | | 2159 | | | | 2883 | 2947 |
| Water bullalo | 1105 | 1192/1208 | 1455 | 1580 | | | 2131 | | | | 2883/2899 | 3059/3075 |
| Dog/wolf | 1105 | 1226 | 1453 | 1566 | | | 2131 | 2216 | 2342 | | 2853 | 2999 |
| Bear: brown, black, polar | 1105 | 1233 | 1453 | 1566 | | | 2163 | 2216 | 2342 | | 2853 | 2957 |
| Lion | 1105 | 1223 | 1453 | 1566 | | | 2147 | 2216 | 2342 | | 2853 | 2999 |
| Lynx | 1105 | 1223 | 1453 | 1566 | | | 2147 | | | | 2853 | 2957 |
| Human | 1105 | 1235 | 1477 | 1580 | | | 2115 | 2216 | | | 2869 (2885?) | 2957(w) |
| Mustelidae (Badger) | 1105 | 1221 | 1453 | 1566 | | 1962 | 2147 | | 2344? | 2753 | 2853 | 2999 |
| Mustelidae (Ermine) | 1105 | 1235? | 1453 | 1566 | | 1953 | 2147 | 2199/2216 | 2342/2348 | 2753 | 2853 | 2999 |
| Mustelidae (Fisher) | 1105 | 1189/1205 | 1453 | 1566 | | | 2147 | 2199/2216 | 2342/2348 | 2753 | 2853 | |
| Mustelidae (Martin) | 1105 | 1235 | 1453 | 1566 | | | 2147 | 2216 | 2342/2348? | 2753? | 2853 | 2999 |
| Mustelidae (Mink) | 1105 | 1219/1230/1235 | 1453 | 1566 | | 1953 | 2147 | 2199/2216 | 2342/2348 | 2753 | 2853 | 2999 |
| Mustelidae (River otter) | 1105 | 1235 | 1453 | 1570? | | | 2147 | 2199/2216 | 2332/2348 | 2753? | 2853 | 2999 |
| Mustelidae (Sea otter) | 1105 | 1235 | 1453 | 1566 | | | 2147 | 2199/2216 | 2332/2348 | 2753? | 2853 | 2999 |
| Mustelidae (Weasel) | 1105 | 1235? | 1453 | 1566 | | 1953 | 2147 | 2216 | 2342/2348? | 2753 | 2853 | 2999 |
| Mustelidae (Wolverine) | 1105 | 1219/1230/1235 | 1453 | 1566 | | | 2147 | 2216 | 2342/2348 | 2753 | 2853 | 2999 |
| Sciuridae (all) | 1105 | | 1453 | 1576/1578 | | | 2143 | | | | 2883 | |
| Sciuridae (Bangs flying squirrel) | 1105 | 1182/1198/1207 | | | | 1934 | | 2232 | | 2767 | | 2999 |
| Beaver (North American) | 1105 | 1177/1193 | 1427 | 1593? | | 1964? | 2129 | 2199/2216 | 2348? | 2743 | 2883 | 2999 |
| Cricetidae (Muskrat) | 1105 | 1182? | 1453 | 1552 | | 1947? | 2143 | 2216 | | 2743 | 2883 | 3059? |

Table 4. PMF markers used in this work. See Methods and materials/Table of Markers above for details.

## 2.8 Methods and Materials/PMF Consumables and Equipment

| DIGESTION CONSUMABLES | |
|---|---|
| Company: VWR | |
| Item | VWR Cat. # |
| Trifluoroacetic acid, EMD Chemicals, 100 g | EM-TX1275-1 |
| Formic acid AR* ACS Grade, 120 mL | MK259202 |
| Ammonium bicarbonate, Baker Analyzed Reagent, 500 g | JT3003-1 |
| pH Indicator strip, EMD, 0-14, pkg 100 | EM-9590-3 |
| Pipet tips, Gilson-Style, Axygen No. RFL-300-C, 0.5-10 uL, cs of 5 | 89029-921 |
| Pipet tips, Universal Fit, Axygen No. RFL-222-C, 1-200 uL, pack of 960 | 89029-919 |
| Microcentrifuge tubes, 0.65 mL, natural, pack of 1000 | 89000-010 |
| Microcentrifuge tubes, 1.5 mL, natural, pack of 500 | 89000-028 |
| | |
| Company: Sigma Aldrich | |
| Item | Sigma Aldrich Number |
| Acetonitrile, Chromsolve Plus >99.9%, 1L | 34998-1L |
| HPLC water, Chromasolve, 4L | 270733-4L |
| Methanol, Chromasolve, >99.9%, 2L | 34860-2L-R |
| Iodoacetamide 5 g | I6125-5G |
| alpha-cyano-4-hydroxycimmamic acid, ≥ 99% (CHCA) | 70990-1G-F |
| | |
| Company: Promega | |
| Item | Promega Number |
| Promega, Sequencing grade modified trypsin, 100 ug, lyophilized, | V5111 |
| | |
| Company: Millipore | |
| Item | Millipore Number |
| ZipTip® Pipette Tips, 0.6 µL C18 resin | ZTC18S096 |
| | |
| TYPICAL EQUIPMENT | |
| Item | VWR Cat. # |
| Micro centrifuge, VWR Mini | 37000-700 |
| Vortex Mixer, VWR Signature, Digital | 14005-824 |
| Thermomixer, Eppendorf # 022670000 | 21516-170 |
| Freezer, under counter, Northland-Marvel 4.5 cu ft | VF-4CAF |
| Bench top clean hood, Filtco, dist. by Air Science USA (optional) | VLF-24 |
| Analytical Balance, Ohaus #AV114C | 87000-802 |

Table 5. Supplies and equipment used for peptide mass fingerprinting.

## 2.9 Methods and Materials/Reference Materials

Table 6 lists the common name and taxon for reference materials analyzed as part of this work. These, in addition to markers (table 4), are the basis of material identification.

| Common name | Taxon | Common name | Taxon |
|---|---|---|---|
| Armadillo (nine banded) (2) | Dasypus novemcinctus | Pig (ferel) | Sus scrofa |
| Baboon (olive) | Papio anubis | Pika (collared) (2) | Ochotona collaris |
| Badger (American) (3) | Taxidea taxus taxus | Porcupine (common) | Erethizon dorsatum |
| Bear (black) (4) | Ursus americanus | Porpoise (dall) | Phocoenoides dalli |
| Bear (brown) (3) | Ursus arctos horribilis | Porpoise (harbor) | Phocoena phocoena |
| Bear (polar) (2) | Ursus maritimus | Possum (brush tailed) | Trichosurus vulpecula |
| Beaver (North American) (3) | Castor canaadensis | Prairie dog | Cynomys ludovicianus |
| Bobcat (2) | Lynx rufus | Pronghorn (2) | Antilocapra americana |
| Buffalo (4) | Bison americanus | Rabbit (black tailed jackrabbit) | Lepus californicus |
| Buffalo (Cape) | Syncerus caffer | Rabbit (brush) (2) | Sylvilagus bachmani cinerascens |
| Caribou (5) | Rangifer tarandus | Rabbit (common) (2) | Oryctolagus cuniculus |
| Caribou (Grant's) | Rangifer tarandus granti | Rabbit (pygmy) | Brachylagus idahoensis |
| Cattle | Bos taurus | Rabbit (snowshoe) (2) | Lepus americanus |
| Chinchilla | Chinchilla lanigera ? | Raccoon (3) | Procyon otor lotor |
| Cougar (2) | Puma concolor | Rat (bushy-tailed wood) (2) | Neotoma cinera orolestes |
| Coyote (2) | Canis latrans latrans | Rat (Norway) | Rattus norvegicus |
| Deer (black tailed) | Odocoileus hemionus columbianus | Reedbuck | Redunca arundinum ? |
| Deer (California mule) (2) | odocoileus hemionus californicus | Sea lion (Steller) (2) | Eumetopias jubatus |
| Deer (mule) (2) | Odocoileus hemionus scaphinotus | Seal (bearded) (2) | Erignathus barbatus |
| Deer (Sitka black tailed) | Odocoileus hemionus sitkensis | Seal (harbor) (3) | Phoca vitulina |
| Deer (white tailed) (2) | Odocoileus virginianus | Seal (northern elephant) (2) | Mirounga angustirostris |
| Duiker (common) | Sylvicapra grimmia | Seal (northern fur) (2) | Callorhinus ursinus |
| Elk (2) | Cervus elaphus nelsoni (Elk) | Seal (Pacific bearded) | Erignathus barbatus |
| Elk (Tule) | cervus elaphus nannodes | Seal (ribbon) (2) | Histriophoca fasciata |
| Ermine (2) | Mustela erminea kadiakensis | Seal (ribbon) (2) | Histriophoca fasciata |
| Fisher (2) | Martes pennanti | Seal (ringed) (2) | Pusa hispida |
| Fox (Arctic) (3) | Alopex lagopus | Seal (spotted) | Phoca largha |
| Fox (gray) (3) | Urocyon cinereoargenteus | Sheep (dall) (2) | Ovis dalli |
| Fox (red) (4) | Vulpes vulpes | Shrew (common) | Sorex cinereus cinereus |
| Geoffroys cat | Leopardus geoffroyi | Shrew (tundra) (2) | Sorex tundrensis |
| Goat (mountain) (3) | Oreamnos americanus columbiae | Skunk (spotted) | Spilogate putoris |
| Gopher (Camus pocket) (2) | Thomomys bulbivorus | Skunk (striped) (3) | Mephitis mephitis spissigrada |
| Gopher (northern pocket) (2) | Thomomys talpoides bullatus | Springbok | Antidorcas marsupialis |
| Gopher (plains pocket) | Geomys bursarius | Squirrel (arctic ground) (2) | Spermophilus parryii |
| Grysbok | Raphicerus melanotis | Squirrel (Bangs flying) (2) | Glaucomys sabrinus bangsi |
| Horse (2) | Caballus caballus | Squirrel (eastern gray) | Sciurus carolinensis |
| Human | Homo sapiens | Squirrel (red) (2) | Tamiasciurus hudsonicus |
| Impala | Aepyceros melampus | Squirrel (western gray) (2) | Sciurus griseus griseus |
| Kangaroo (red) | Macropus rufus | Vole (red-backed) (2) | Clethrionomys rutilus dawsoni |
| Lama | Lama huanachus glama | Walrus (3) | Odobenus rosmarus rosmarus |
| Lechwe | Kobus leche | Warthog | Phacochoerus africanus |
| Lemming (collared) (2) | Dicrostonyx groenlandicus | Weasel (least) (2) | Mustela nivalis eskimo |
| Lynx (Canada) (2) | Lynx canadensis canadensis | Weasel (long tailed) | Mustela frenata |
| Marmot (hoary) (2) | Marmota caligata oxytona | Whale (beluga) | Delphinapterus leucas |
| Marmot (yellow-bellied) (2) | Marmota flaviventris avara | Whale (blue) | Balaenoptera musculus |
| Martin (pine) (3) | Martes americana americana | Whale (fin) | Balaenoptera physalus |
| Mink (American) (3) | Neovison vison | Whale (gray) | Eschrichtius robustus |
| Moose (2) | Alces alces americana | Whale (northern right) | Eubalaena japonica |
| Mouse (deer) (2) | Peromyscus maniculatus | Whale (orca) | Orcinus orca |
| Mouse (house) (2) | Mus musculus | Whale (sei) | Balaenoptera borealis |
| Mouse (measow jumping) (2) | Zapus hudsonius acadicus | Wildebeest (blue) | Connochaetes taurinus |
| Musk Ox | Ovibos moschatus | Wolf (British Columbian) | Canis lupus columbianus |
| Muskrat (2) | Ondatra zibethicus | Wolf (gray) (3) | Canis lupus |
| Opossum | Didelphis virginiana | Wolverine (3) | Gulo gulo |
| Otter (river) | Lontra canadensis | Woodchuck (2) | Marmota monax petrensis |
| Otter (sea) (3) | Enhydra lutris | Zebra (common) | Equus quagga |

Table 6. List of reference samples with PMF's. The number of individual samples is indicated by (#).

# 3. Results and Discussion

## 3.1 Results and Discussion/Summary of Object Sample Analyses

Peptide Mass Fingerprinting was used to survey a selection of late-18th – early-20th century hide, skin, and inner membrane (gut) objects from the Peabody Museum's collection, two archaeological samples (800 BC – AD 400) from the Peabody Museum, and approximately 200 reference samples obtained from several different collections.

In selecting samples, the project focus was primarily on skin-constructed Alaska Native and Native American objects from coastal Alaska, the Northwest Coast, and the High Plains. In total, 449 samples from 111 objects were analyzed. Tabulated results are shown in table 7.

Only 38 of the 449 samples (8.5%) in the entire collection remain unidentified. These materials are most likely avian or fish species, based on visual comparison to appropriate reference spectra, MASCOT[16] database search results, and the absence of familiar mammalian markers in the spectra. Because of the mammalian focus of this project and the lack of non-mammalian references in the database, the unknown samples could not be further analyzed. Future work is needed to extend the database to these underrepresented sources to provide a more complete understanding of materials usage.

It should be noted that four different researchers, three of whom had not previously had experience with this type of analysis and were not familiar with mass spectrometry, obtained the results reported here.

Overall Sample Analyses

- 89% identified to at least to the family level; many to genus and species level.
- 8.5% not identified and most likely fish or bird, which are not in this database.
- 1.6% had NUSO (No Useable Spectrum Observed).
- 1% was not identified exactly because of sample/spectra quality (deer/sheep/goat or eared seal/walrus).
- 30 different mammalian sources were identified either exactly or to a limited group, such as phocini seals within the family of earless seals.
- 19 samples (4.2%) were identified to at least family level by using new, provisional markers discovered in this project.
- 2 sets of archaeological skin fragments were identified.

Sea and Land Mammals

Of the 404 samples identified as sea or land mammals:

- 59% were sea mammals and 41% were land mammals, reflecting the focus on coastal areas for the sampled objects.
- Of the sea mammals, 78% were seals and 22% were walrus and cetaceans.
- Of the seals, 57 % were earless or true seals (phocini, bearded) and 43 % were eared seals (Steller sea lions, northern fur seals).
- Among the land mammals, 46% were caribou with the remaining 54% spread out over 11 different mammals.
- Caribou was very frequently used as a source of sinew.

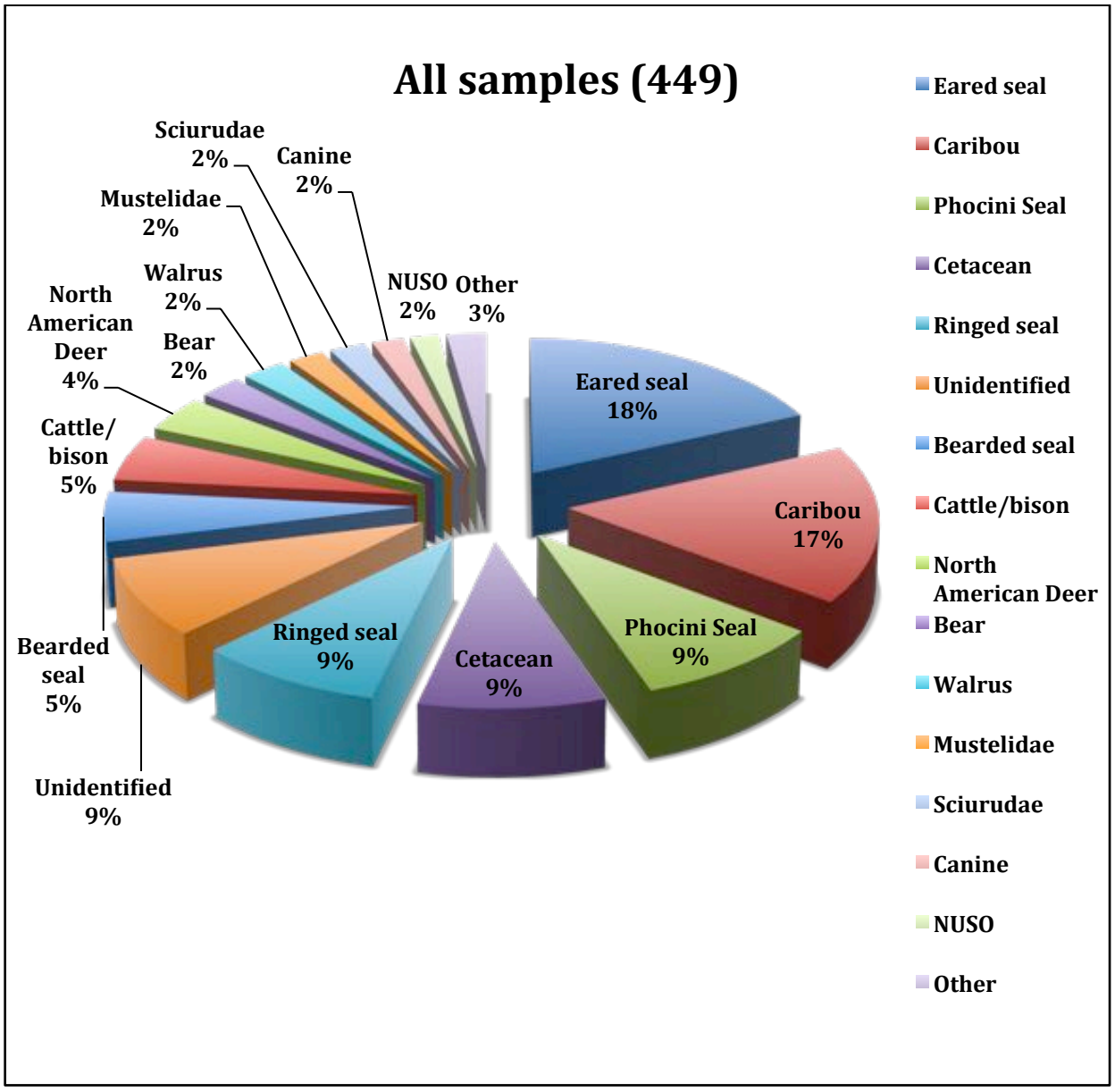


Table 7. Distribution of samples from objects. Unidentified: PMF spectrum obtained but did not match any reference in the database; probably fish or bird. NUSO: No Useable Spectrum Observed, digest was unsuccessful for unknown reasons. Other includes the following samples: Deer/sheep/goat, not determined exactly (3); elk (3); sheep (3); eared seal/walrus, not determined exactly (2); goat (2); muskrat (1).

Cetaceans

Among the 40 cetaceans samples (table 8), all of which were from sinew:

- 68% (27 samples) were identified to species level.
- 32% (13 samples) were identified to a limited group such as orca/white sided dolphin, dolphin, or porpoise.

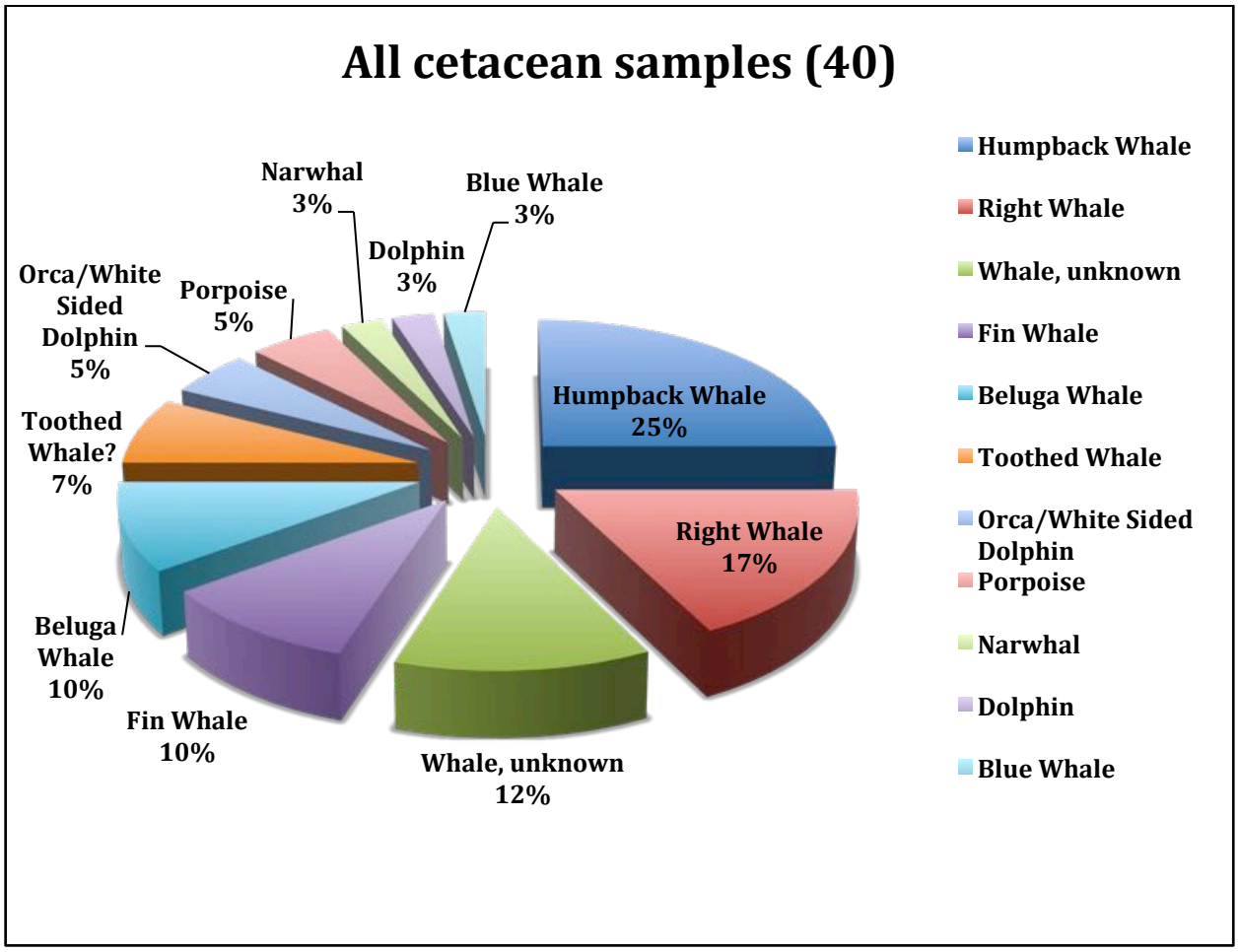


Table 8. Distribution of cetacean samples. Whale, unknown: clearly cetacean based on observed markers but not consistent with any in the database; Toothed whale?: possibly toothed whale based on observed markers, but not consistent with the two toothed whale references in the database.

### 3.2 Results and Discussion/Summary of Reference Sample Analyses

Over 200 new reference samples were gathered from several mammalian collections and analyzed as part of this project. Some of these duplicated existing references, but the majority were new mammals, especially those representing small ground mammals indigenous to the focus areas. These included:

- Leporidae: rabbits and hares.
- Geomyidae: gophers.
- Muridae: rodents including voles and mice.
- Mustelidae: carnivorous mammals including otters, badgers, weasels, martens, ferrets, minks, and wolverines.
- Sciuridae: small-medium-sized rodents including tree squirrels, ground squirrels, chipmunks, marmots, woodchucks, flying squirrels, and prairie dogs.

The last two families, mustelidae and sciuridae, have not previously been documented as references for PMF with the exception of badger, which is included in a prior publication.[12] Among the 449 samples analyzed in this project, 9 were identified as mustelidae and 9 as

sciuridae based on these new references. Table 4 shows provisional markers for these two families and for sub-families of mustelidae. Additional work is needed to verify the provisional markers, particularly LCMSMS analysis to confirm peptide sequences, but that is not part of the present work. The provisional markers are very useful, however, for limiting the number of PMF spectra that might be considered for visual comparison with an unknown. In particular, the D markers in table 4 are quite specific for mustelidae (2147 Da) and sciuridae (2143 Da), significantly narrowing the number of possible sources among the references.

3.3 Results and discussion/Five Examples Using PMF

The identification of collagen-based components in 111 objects from the Peabody Museum has significantly enhanced the collection records for these objects. Little documented information about material origins had previously been available. The following five case studies illustrate the unique capabilities of PMF for obtaining accurate materials information quickly and reliably. The examples include a pair of Yup'ik child's boots, an Alutiiq woman's sewing bag, two archaeological fragments from the Ohio River valley, a Yup'ik kayak model from the Norton Sound area, and two Alaska Native gutskin kayak-clothing items.

3.3.1 Child's boots, Peabody Museum of Archaeology and Ethnology, Museum no. 25-5-10/98129.

Background

A pair of intricately constructed Alaskan Native skin boots was donated to the Peabody Museum in 1925 by John Weare (Harvard Class of 1907) in memory of his father, Charles. Charles Ashley Weare, born September 7, 1852 in Iowa, was one of six directors of the North American Transportation and Trading Company, which provided tools, clothing, provisions, and transportation for miners in the gold fields of Alaska during the late nineteenth century[20,21,22]. The company's steamboats traveled from Seattle to St. Michael Island, by way of the Aleutian Islands, and then went on to mining points along the Yukon River. Charles Ashley Weare is known to have traveled to these regions numerous times during the 1890s, and it was during these trips that he became interested in the indigenous cultures and began to collect objects created by Native Alaskans.[23] After his father's death, John Weare donated his father's collection to the Peabody Museum.

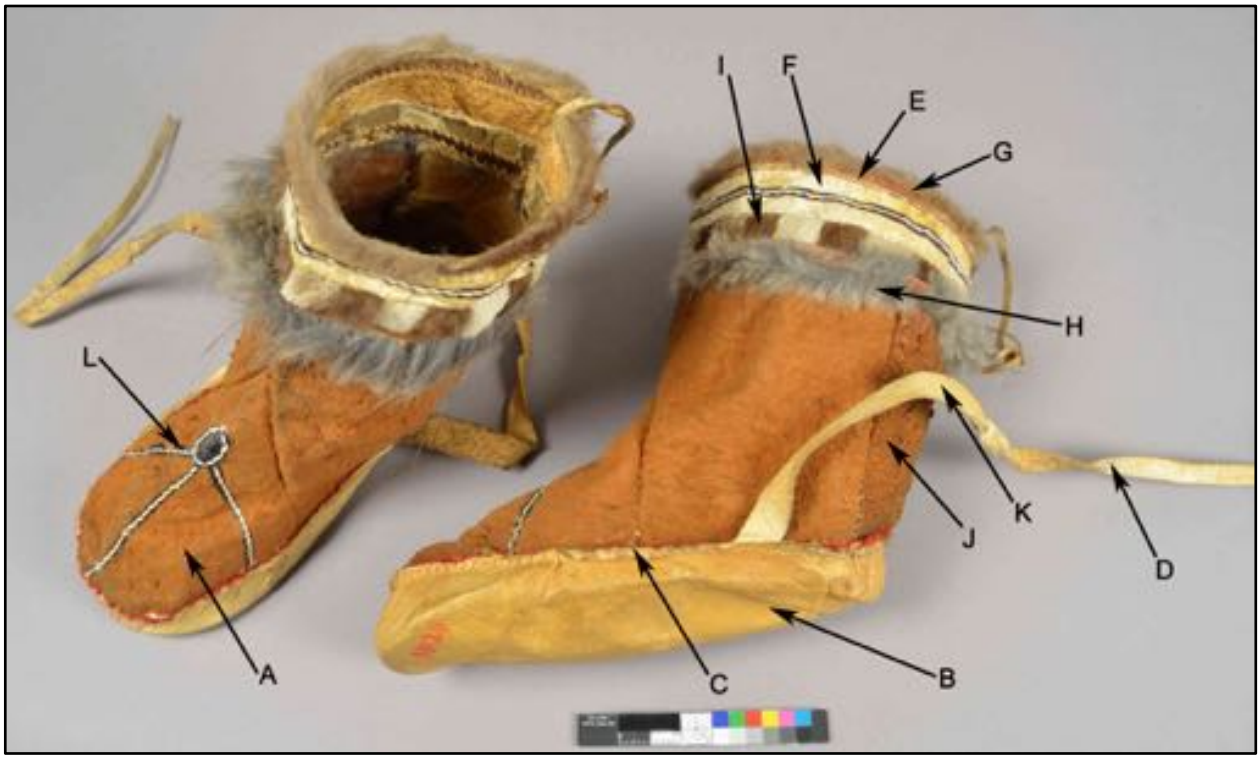


Figure 11. Child's boots showing sampling locations. ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 25-5-10/98129 (digital file# 75720081).

The museum's object record identifies the materials used in the boots as leather, fur, and sinew, and based on visual examination, there appeared to be at least four or five different skin-based components. Subsequent analysis of samples from 12 locations identified five known mammalian sources and one unknown source. The materials are:

- Orange-brown colored skin, main body of boot uppers: A – ringed seal.
- Thick yellowish skin, sole of boot: B – bearded seal.
- Sinew connecting the bottom to the main body: C – caribou.
- Skin from outer end of strap: D – bearded seal.
- Skin from inner end of strap: K – ringed seal.
- Red band at top of boot (just below brown fur): E – ringed seal.
- White band near top of boot (below red band): F – bearded seal.
- Brown fur attached to skin at uppermost part of boot: G – mustelidae family (ermine, mink, or weasel).
- Gray fur attached to skin just below the checkerboard pattern: H – muskrat.
- Brown fur attached to skin, part of checkered pattern: I – caribou.
- Heel panel: J – ringed seal.
- Black and white decoration on toe area: L – unidentified, non-mammalian, possibly fish or avian.

Samples from the child's boots were taken from areas of damage or loss to minimize impact on the objects. Additional information about sampling and sample size can be found under Methods and Materials.

PMF analyses identified five different mammalian material sources for the samples taken from the boots: caribou, bearded seal, ringed seal, muskrat, and mustelidae (ermine, mink or weasel), as well as one unknown source, possibly fish or avian. Additional information about PMF can be found under Methods and Materials.

Analytical results

Figure 12 is the PMF from figure 11, location A, identifying ringed seal as the origin of the orange-red material in the boot uppers. Markers used for the identification are indicated. Ringed seal is a member of the phocini tribe of the phocidae family, the so-called true or earless seals. PMF cannot discriminate among members of phocini (ribbon, spotted, ringed, gray, harbor and harp) with the exception of ringed seal, which can be differentiated from other phocini seals with the (P1) marker at 2232 Da instead of 2216 Da. The G marker at 2957 Da ion is not observed in the PMF but is unnecessary since the A, D and F markers, along with the (P1) marker, uniquely identify ringed seal in the database.

Figure 13 is the PMF from the boot sole (figure 11, location B) identifying bearded seal as the origin of that material. Bearded seal, also a member of the phocidae family, can be differentiated from all other phocidae in the database by its characteristic Cet1 marker at 1121 Da. Bearded seal was also identified as the material in figure 11, locations D and F, the outer end of the strap and the white band at the boot top, respectively.

Figure 14 is the PMF from the sinew used to attach the boot soles to the uppers (figure 11, location C) and is identified as caribou. Among the references in table 4, caribou is the only entry with an A marker at 1166 Da and is easily recognized from its PMF. Caribou was also identified as the material in the checkerboard patterned decorative band at the boot top (figure 11, location I).

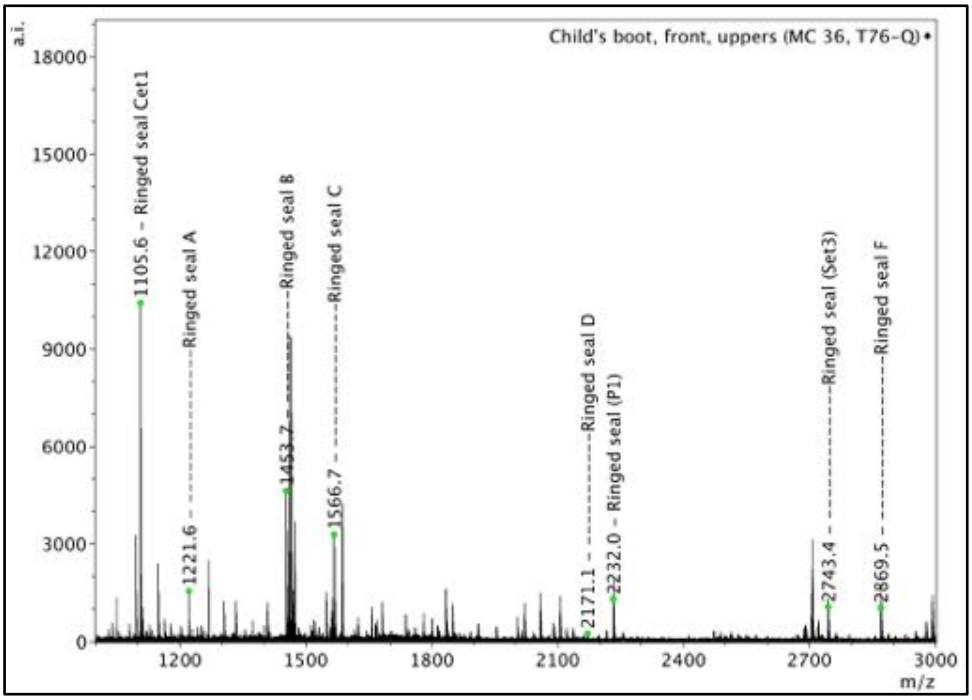


Figure 12. PMF from figure 11, location A: ringed seal.

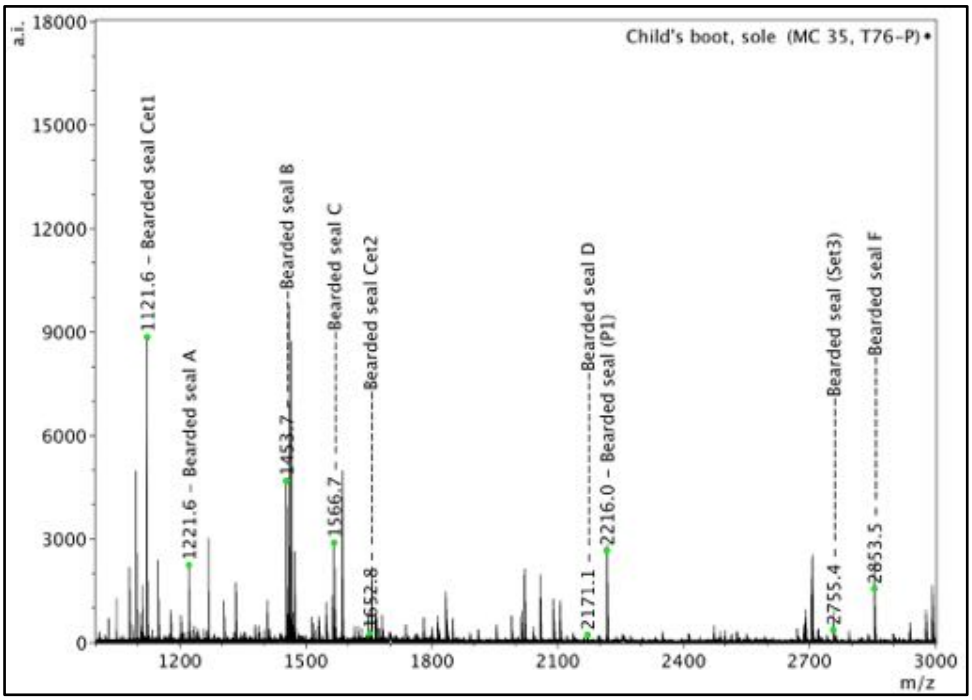


Figure 13. PMF from figure 11, location B (also locations D and F): bearded seal.

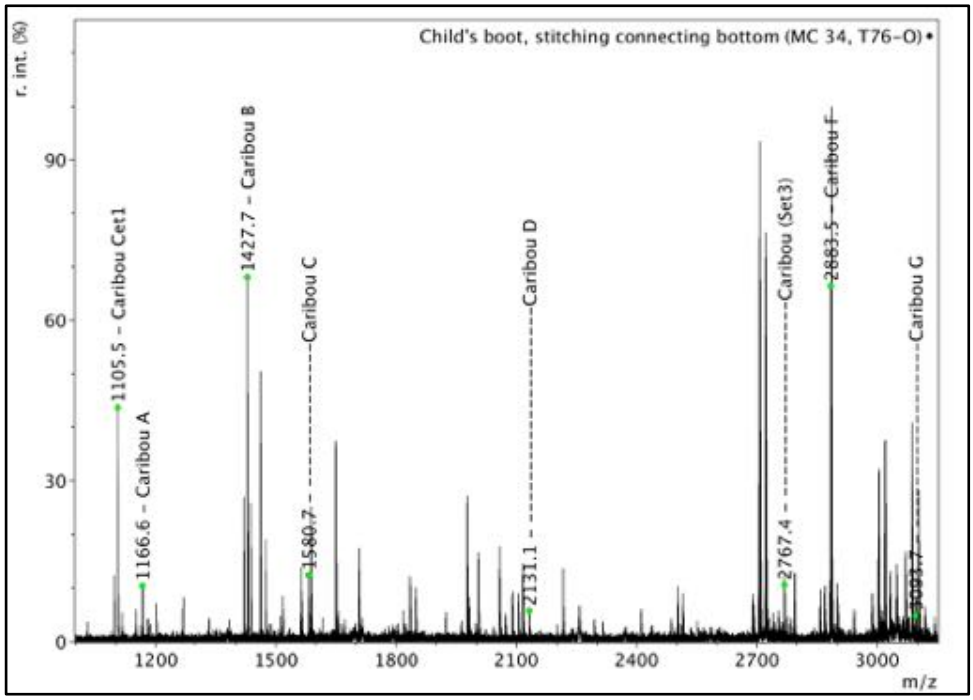


Figure 14. PMF from figure 11, locations C and I: caribou.

The PMF from the skin of the gray fur at the top of the boots (figure 11, location H) did not match any of the published references in table 4; notably absent was a known D marker. We suspected that the 2143 Da ion (figure 15) was the D marker and, among the new reference materials that were analyzed for this project, only sciuridae (small to medium sized rodents including squirrels, chipmunks and marmots) and muskrat (cricetidae) contained that marker (table 4). Among those possibilities, muskrat was identified as the source of the gray fur. The similarity of the gray fur PMF to the muskrat reference PMF, as well as its dissimilarity to other references containing a 2143 Da marker, was used as the basis for the identification. Figure 15 compares a section of the PMF's from the child's boot and the muskrat reference showing the high degree of similarity, including the D marker. The PMF identification of the gray fur was corroborated with polarizing light microscopy (PLM). Several hairs from the gray fur strip were observed with PLM and compared with known hairs from squirrels (red, gray), marmot, and muskrat. The gray fur was verified to be muskrat.

The PMF for the skin of the brown fur at the top of the boot (figure 11, location G) did not match any reference in table 4 but contained an ion at 2147 Da, which we suspected was the D marker. Buckley's table[12] contains a single entry with that marker: badger (mustelidae family). As part of this project, a number of additional mustelidae samples, including ermine, fisher, martin, mink, otter, weasel, and wolverine, were collected and analyzed, and tentative markers have begun to be assigned for this family and several of its sub families (table 4). Based on these assignments, the skin attached to the brown fur has been tentatively identified as mustelidae (ermine, mink or weasel). Figure 16 shows the PMF for the brown fur with markers indicated.

The PMF's for materials in the black and white decoration (figure 11, location L) did not match any markers in the database, and the absence of highly conserved mammalian

markers, especially those at 1095, 1105, 1459, and 2703 Da, was convincing evidence that these materials were not mammalian. The PMF mass lists were searched through MASCOT[16], an on-line database search engine for protein identification, but the results were inconclusive, most likely due to the lack of available sequence information. Based on those results, however, it is believed these materials are of avian or fish origin.

Summary of results

The main boot body material is ringed seal, as are the reddish and white bands at the top of the boots, the heel panels and the inner part of the straps. The soles, the outer part of the yellowish straps, and the white band at the top are bearded seal. The sinew stitching joining the tops with the soles and the brown fur in the checkered pattern at the top is caribou. The brown fur at the boot top is mustelidae (mink, ermine or weasel), and the gray fur is muskrat. The black and white materials in the toe decoration are unknown, possibly avian or fish.

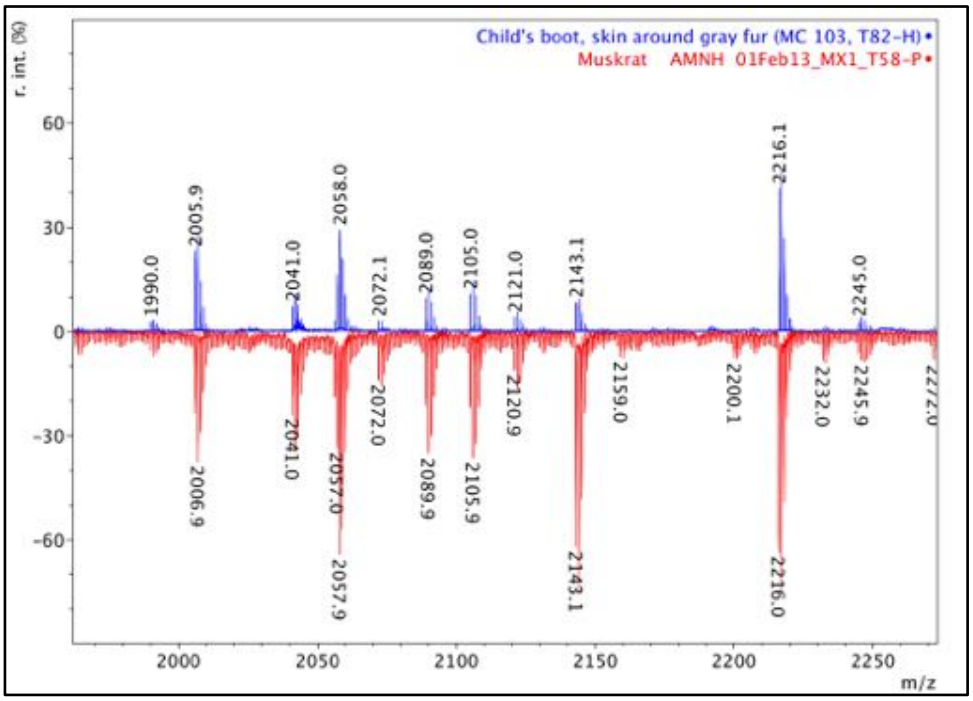

Figure 15. PMF (partial) from figure 11, location H: muskrat.

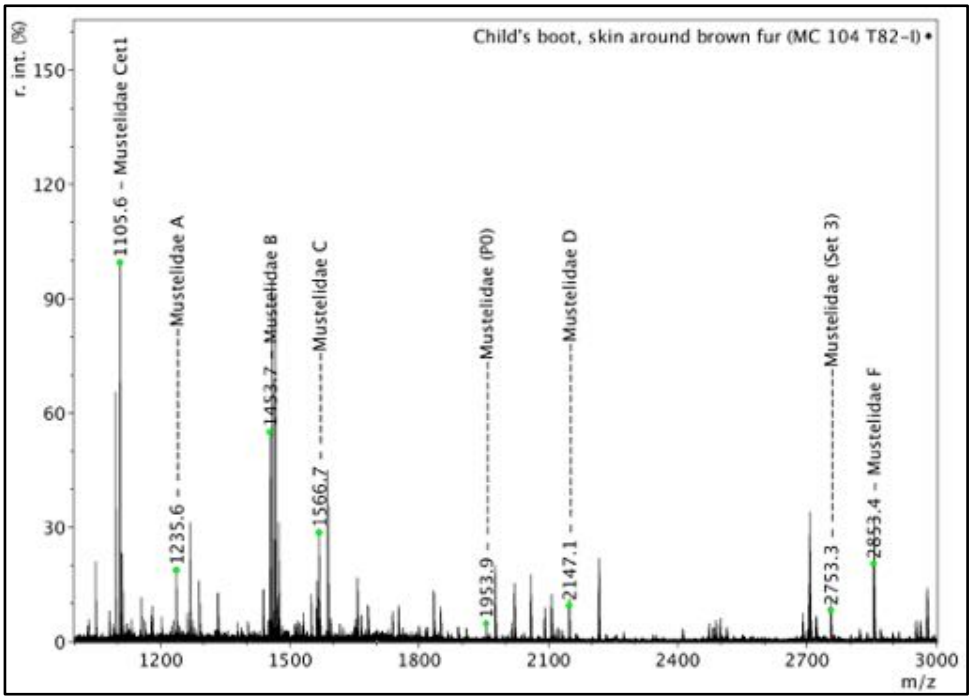


Figure 16. PMF from figure 11, location G: mustelidae (ermine, mink or weasel).

3.3.2 Woman's embroidered skin sewing bag (*kakiwik*), Peabody Museum of Archaeology and Ethnology, Museum no. 11-2-10/83860.

Background

A coastal Alutiiq/Sugpiag skin sewer created this intricately decorated late-19th century *kakiwik*. It was donated to the Peabody Museum in 1911 by Dr. William McM. Woodworth (Harvard Class of 1889), who had earlier collected it on Woody Island (Tangirnaq) located about three miles east of Kodiak Island, Alaska. The *kakiwik* is constructed of skin and esophagus and sewn with sinew. The Alutiiq consultants to the Peabody in 2012 informed us that it was most likely of *wiinaq*, or sea lion (an eared seal)[24]. The bag is constructed with a rounded upper flap and two separate front pieces positioned thus to function as two distinct pouches. Four stitched sections of unpainted skin form the rear side of the bag. The face side features painted strips appliquéd and embroidered with various materials including caribou hair. The long braided cord at the top is constructed of either sinew or twisted gut. This cord was used to hold and tie the bag in a rolled up state for compact storage and for keeping the inside contents secure.

Examination of the bag suggested the presence of several different materials. Analysis of samples from six locations identified four known mammalian materials and two different, unknown, non-mammalian materials. The materials were:

- Sinew stitching and strap: A – blue whale.
- Skin, top band: B – phocini seal.
- Inner red band in red and black edge decoration: C – unknown, non-mammalian.
- Main body material: D – eared seal.
- Black coated skin: E – caribou.
- Skin with bright red coloring: F – unknown, non-mammalian.


Figure 17. Woman's embroidered sewing bag showing sampling locations. Overall dimensions 44.5 x 22.6 x 0.8 cm. ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 11-2-10/83860 (digital file# 75720080).

Samples from the sewing bag were taken from areas of damage or loss to minimize impact on the object. Additional information about sampling and sample size can be found under Methods and Materials.

PMF analyses of the sewing bag materials identified four different mammalian sources: blue whale, phocini seal, eared seal and caribou, and two different, unknown sources, possibly either fish or avian. Additional information about PMF can be found under Methods and Materials.

Analytical results

Figure 18 is the PMF from the sinew (figure 17, location A) identifying blue whale as the source of that material. Blue whales are members of the infraorder cetacea, which are marine mammals including whales, dolphins and porpoises. PMF's of cetaceans are characterized by a Cet1 ion at either 1063 or 1079 Da and an A ion at 1205 Da (table 9). Among the 22 cetaceans in table 9, ten whales, including blue whales, can be identified uniquely. The remaining cetaceans fall into four groups. The shading in table 9 illustrates how the markers are used for identification. Thus, 13 entries have identical Cet1 and A markers (1079 and 1205 Da), and two entries (Risso's dolphin, pilot whale and false killer whale) and (bottlenose and Sowerby's whale) are eliminated. Considering the B marker, four additional entries are eliminated (narwhal, beluga whale, minke whale, and sei whale). Similarly, considering the C, Cet2, and D markers, all entries except blue whale are eliminated. It is fortuitous that cetaceans have a high diversity of markers allowing the unique identification of many whales.

Figure 19 is the PMF for the material from the top band of the sewing bag closure (figure 17, location B) and identifies that material as phocini seal. Phocini seals are members of the phocini tribe of the phocidae family, the so-called true or earless seals. Markers do not differentiate among phocini seals with the exception of ringed seal, which is characterized by a (P1) marker at 2232 Da, whereas all other phocini seals in the database have the (P1) marker at 2216 Da. Thus, the material in figure 17, location B may be ribbon, spotted, gray, harbor or harp seal, but *not* ringed seal. Note in figure 19 that neither the D marker (2171 Da) nor the G marker (2957 Da) is observed. However, in this case, those markers are not needed because the combination of the other markers, particularly (P1) and F, uniquely identify phocini seals among the mammals in the database.

Figure 20 is the PMF from the main body material (figure 17, location D) and identifies that material as otariidae, the so-called eared seals. Steller sea lions and northern fur seals represent otariidae in the database, and these are not distinguishable by PMF. The G marker is not observed but is unnecessary for the identification since the combination of the other markers, particularly D (2121 Da) uniquely identifies eared seals among the mammals in the database.

Figure 21 is the PMF from the black painted skin on the inside of the sewing bag closure (figure 17, location E) and the horizontal stripes on the body of the bag. This material is identified as caribou, which has a unique A marker (1166 Da) and can readily be distinguished from all other mammals in the database.

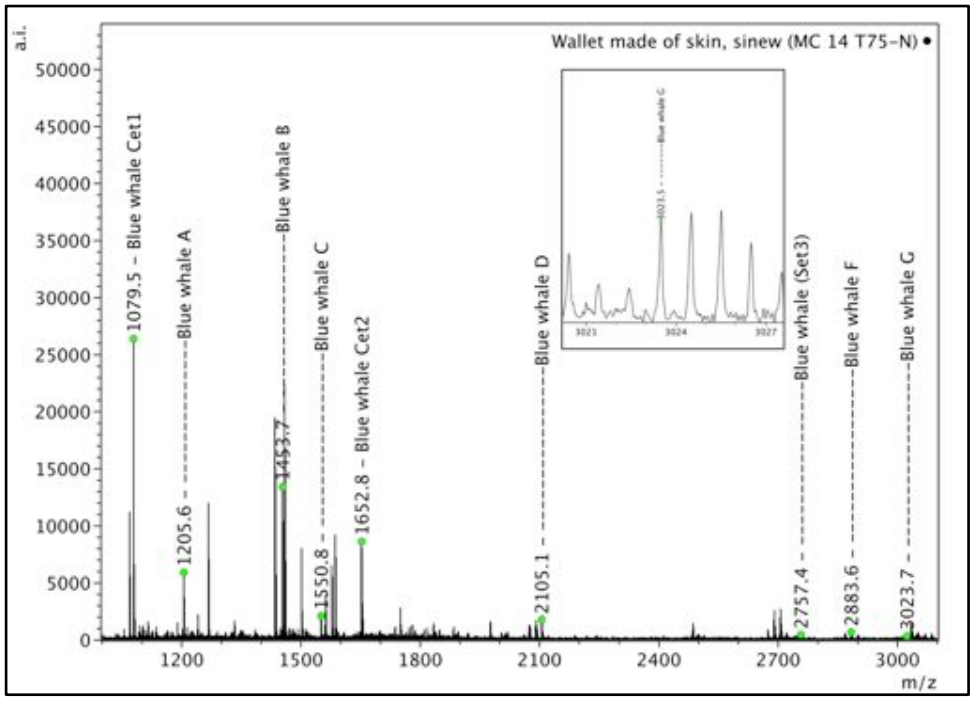

Figure 18. PMF from figure 17, location A: blue whale. The inset shows the G marker.

| | Cet1 | (A) | (B) | (C) | Cet2 | (D) | (P1) REF | (Set3) REF | (F) | (G) |
|---|---|---|---|---|---|---|---|---|---|---|
| Common / Bottlenose / White-beaked / Euphrosyne dolphin | 1079 | 1205 | 1453 | 1566 | 1638 | 2119 | 2225 | 2767 | 2883 | 3023 |
| Risso's Dolphin / Pilot whale / false killer whale | 1063 | 1205 | 1453 | 1566 | 1638 | 2119 | 2225 | 2767 | 2883 | 3023 |
| Orca / White-sided dolphin | 1079 | 1205 | 1453 | 1566 | 1652 | 2119 | 2225 | 2767 | 2883 | 3023 |
| Porpoise | 1079 | 1205 | 1453 | 1550 | 1652 | 2119 | 2225 | 2767 | 2883 | 3023 |
| * Narwhal | 1079 | 1205 | 1443 | 1550 | 1652 | 2089 | 2225 | 2777 | 2883 | 3051 |
| * Beluga whale | 1079 | 1205 | 1443 | 1550 | 1652 | 2121 | 2225 | 2777 | 2883 | 3051 |
| * Sperm whale | 1079 | 1205 | 1453 | 1550 | 1652 | 2133 | 2225 | 2747 | 2883 | 3039 |
| Bottlenose / Sowerby's whale | 1063 | 1205 | 1441 | 1550 | 1638 | 2091 | | | 2883 | 3023 |
| * Minke whale | 1079 | 1205 | 1441 | 1566 | 1652 | 2135 | 2225 | 2757 | 2883 | 3023 |
| * Fin Whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | 2757 | 2883 | 3023 |
| * Humpback whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | 2777 | 2869 | 3023 |
| * Blue whale | 1079 | 1205 | 1453 | 1550 | 1652 | 2105 | | 2757 | 2883 | 3023 |
| * Gray whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | | 2899 | 3023 |
| * Sei whale | 1079 | 1205 | 1441 | 1550 | 1652 | 2135 | | | 2883 | 3023 |
| * Right whale | 1079 | 1205 | 1453 | 1566 | 1682 | 2135 | 2225 | 2789 | 2883 | 3023 |

Table 9. PMF markers used to identify cetaceans. The shading illustrates the process of successively eliminating entries to arrive at blue whale as the source of the sinew.

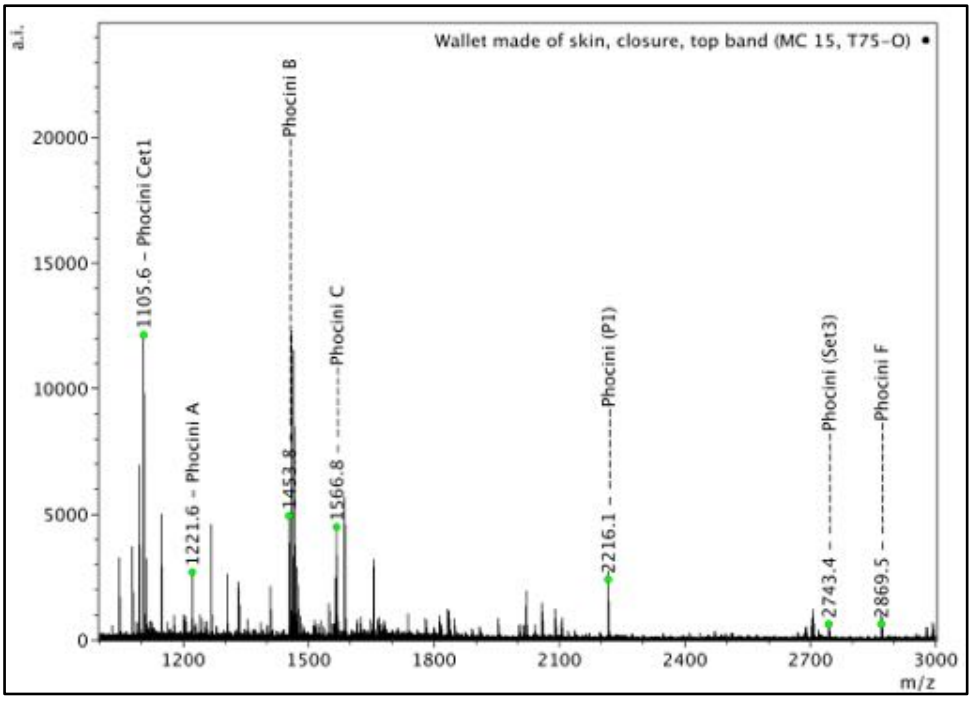


Figure 19. PMF from figure 17, location B: phocini seal (ribbon, spotted, gray, harbor or harp).

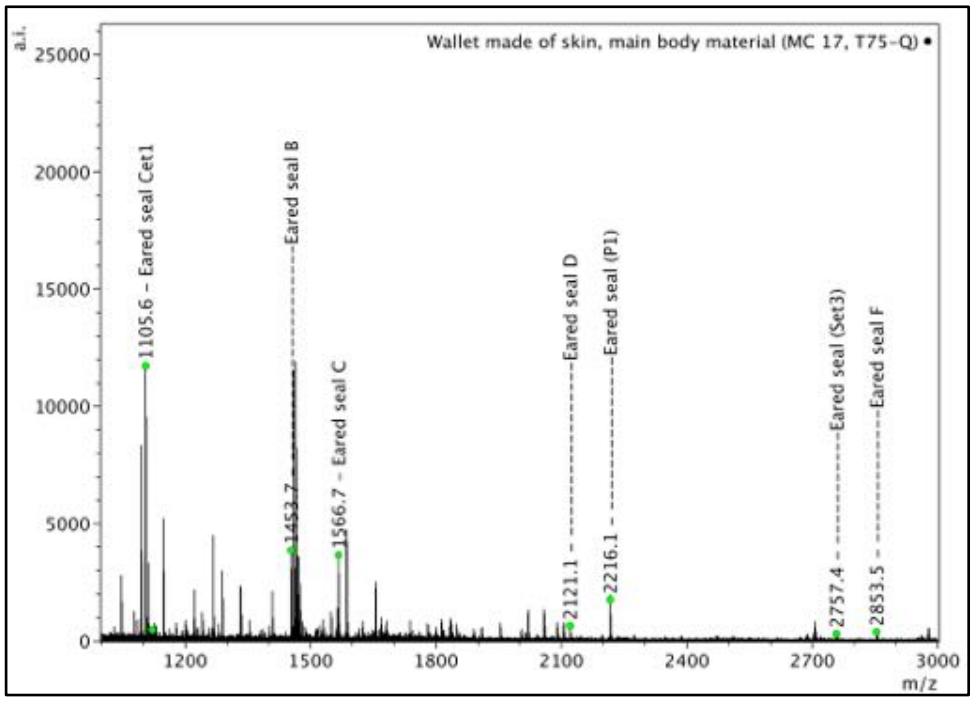


Figure 20. PMF from figure 17, location D: eared seal.

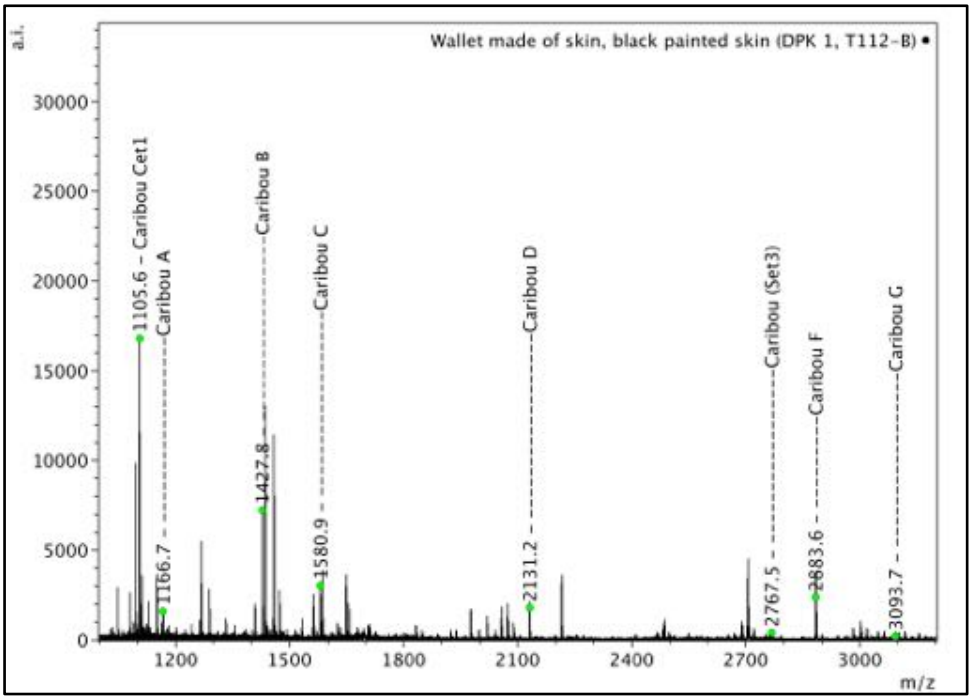


Figure 21. PMF from figure 17, location E: caribou.

Figure 22 shows PMF's of the two unidentified materials from the sewing bag (figure 17, locations C and F). The spectra in figure 22 are very dissimilar indicating that they come from different sources. In addition, neither spectrum shows highly conserved mammalian marker ions at 1095, 1105, 1267, 1459 Da, suggesting that these materials are not mammalian, but possibly avian or fish for which there are currently no references.

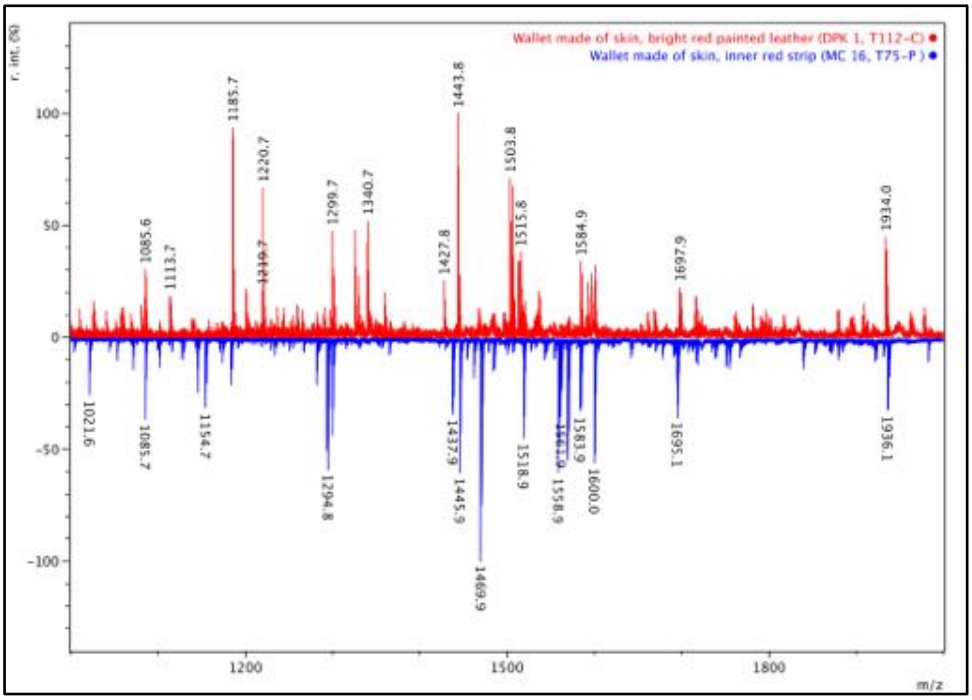


Figure 22. PMF's from figure 17, locations C (blue, lower), F (red, upper) showing dissimalrity and lack of mammalian markers at 1095, 1105, 1267 and 1459 Da.

Summary of results

The main body of this woman's sewing bag is eared seal, the top edge of the closure is phocini seal, and the sinew is blue whale. The black painted skin on the inside of the closure and the black horizontal decoration is caribou. Two red-painted elements, the inner red band of the edge binding and the bright red skin around the black horizontal stripes, are different, unknown materials that are likely not mammalian but possibly avian or fish.

3.3.3 Two archaeological fragmentary objects.

- Buckskin fragments, Peabody Museum of Archaeology and Ethnology, Museum no. 07-65- 10/72842.
- Leather fragments with bead impressions, Peabody Museum of Archaeology and Ethnology, Museum no. 76-6-10/8945.

Background

The archaeological fragments (72842) shown in figure 23 were collected by Dr. Samuel C. Hildreth at a site in Washington County, Ohio dating to the Middle Woodland/pre-Columbian period (200 BC – AD 400). The fragments are described in the museum's object record as "buckskin found between plates of [an] ear ornament," although there is no indication of how the identification of buckskin was determined. The sample taken from the largest piece was quite hard/brittle with a few short fibers extending from its edge. The material was identified as North American deer.

The leather fragments (8945) with bead impressions shown in figure 24 were found in the School House Mound, a site in Athens County, Ohio, dated to 800 – 100 BC[25]. Two samples were taken from the fragments as indicated. This material was also identified as North American deer.


Figure 23. The largest fragment of these "buckskin" fragments was sampled for analysis. ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 07-65-10/72842 (digital file# 75720076).

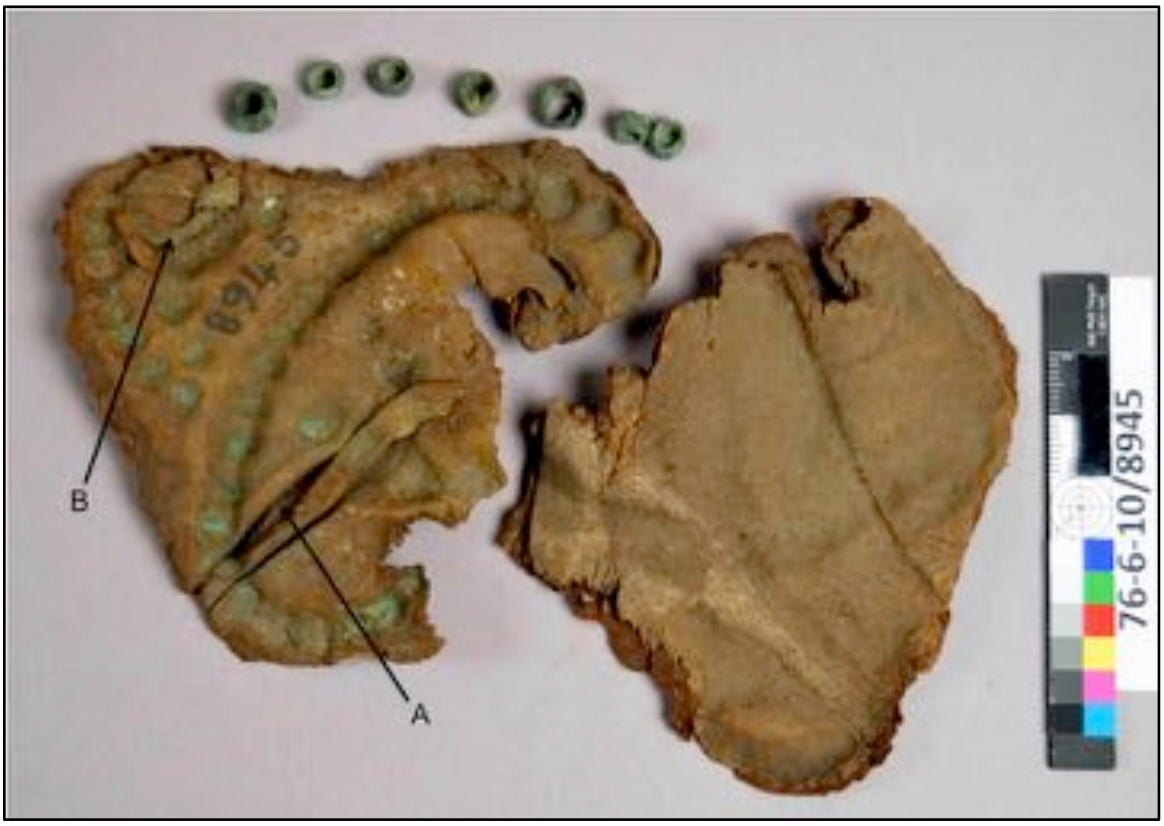


Figure 24. Leather fragments. Samples were taken from inside the folded areas (A) and from the fringe (B). ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 76-6-10/8945 (digital file# 75720075).

The materials in the buckskin (72842) and leather (8945) samples were identified with PMF. Published mammalian markers (table 4, black entries) were used to identify these samples. Markers for closely related sheep, goat, North American deer, elk and Old World deer (red and fallow deer), and roe deer are given in table 10 (a subset of table 4). Additional information about PMF can be found under Methods and Materials.

Both sets of samples yielded good quality spectra, especially considering their age and condition. In both cases, however, ions above 2900 Da were weak and not well resolved making it difficult to identify the G marker, which is critical for differentiating among deer, sheep and goats (table 10). To overcome this difficulty, samples were fractionated using ZipTips® according to Buckley's method[15] and in each case the G marker could then be identified unambiguously. ZipTip® fractionation involves the step-wise elution of a sample adsorbed onto a small bed of chromatographic support in a pipette tip using solvents of increasing strength. The eluted fractions are then analyzed separately by MALDI. Additional information about ZipTip® fractionation can be found in Methods and Materials.

| | Cet1 | (A) | (B) | (C) | (D) | (P1) REF | (Set3) REF | (F) | (G) |
|---|---|---|---|---|---|---|---|---|---|
| Sheep/Pronghorn | 1105 | 1180/1196 | 1427 | 1580 | 2131 | 2199 | 2767 | 2883 | 3033 |
| Goat | 1105 | 1180/1196 | 1427 | 1580 | 2131 | 2199 | 2767 | 2883 | 3093 |
| North American deer ( mule, Sitka, whitetail) | 1105 | 1180/1196 | 1427 | 1580 | 2131 | 2199 | 2767 | 2883 | 3059 |
| Elk, Old World deer (red, fallow) | 1105 | 1180/1196 | 1427 | 1550 | 2131 | 2199 | 2767 | 2883 | 3033 |
| Roe Deer | 1105 | 1180/1196 | 1427 | 1550 | 2131 | 2199 | 2769 | 2883 | 3059 |

Table 10. PMF markers for sheep, goat, North American deer, Old World deer, and roe deer.

Figure 25 is the PMF from the buckskin fragment (72842). The main spectrum shows all the markers for deer except G. The inset shows portions of both the original spectrum and the improved spectrum from the ZipTip® fraction, confirming the G marker at 3059 Da. With the G ion unambiguously defined, this material can be identified as North American deer and distinguished from sheep and goat (table 10). Note also in table 10 that North American deer can be differentiated from elk and Old World deer based on the C marker at 1550 Da and, within that group, roe deer can be uniquely identified with the (Set3) and G markers.

The PMF's from the leather fragments (8945) were also reasonably good spectra considering sample age and condition but, as with the buckskin fragments, the high mass part of the spectrum was weak and poorly resolved thus obscuring the G ion. The partial spectra in figure 26 from each of the leather fragment samples show the data quality in the region where G ions are located. None of the anticipated G markers could be observed at a signal-to-noise ratio of at least 3, the criterion for marker detection. ZipTip® fractionation was again used to improve the spectra and enable the G marker to be identified unambiguously. Figure 27 shows the PMF from one leather fragment and, in the inset, the G ion region from the original spectrum and the ZipTip® fraction for one of the fragment samples confirming the G marker as 3059 Da. The second fragment sample gave identical results, confirming the identity of the material as North American deer.

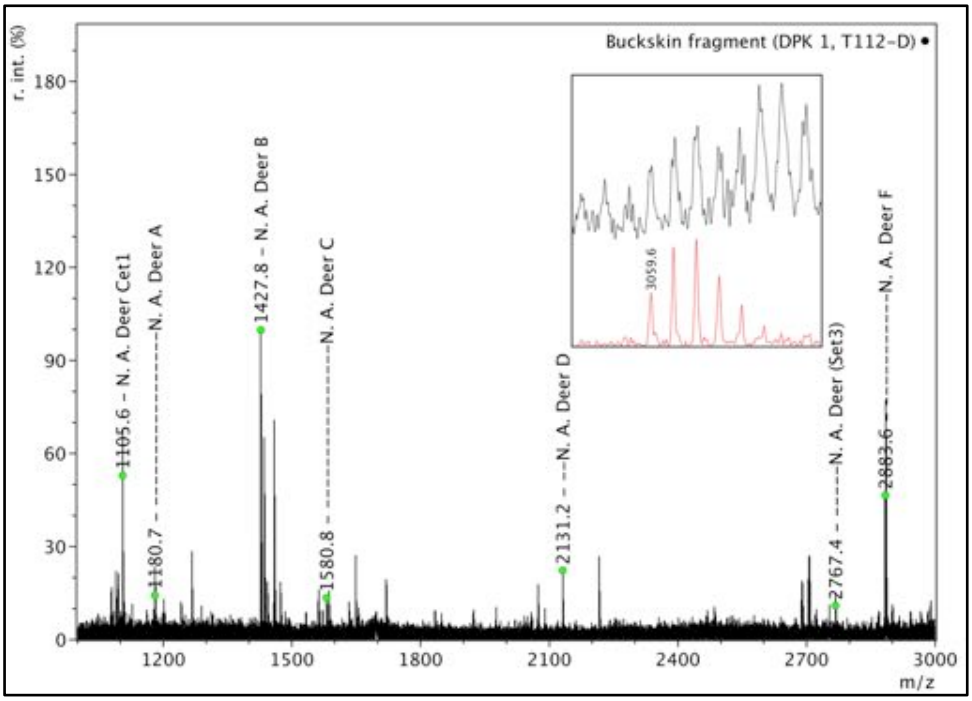


Figure 25. PMF from the buckskin fragment (72842) with markers for North American deer indicated. The inset shows a portion of the spectrum from the original sample (upper, black) and from the ZipTip® fraction (lower, red) clearly indicating the G marker at 3059 Da.

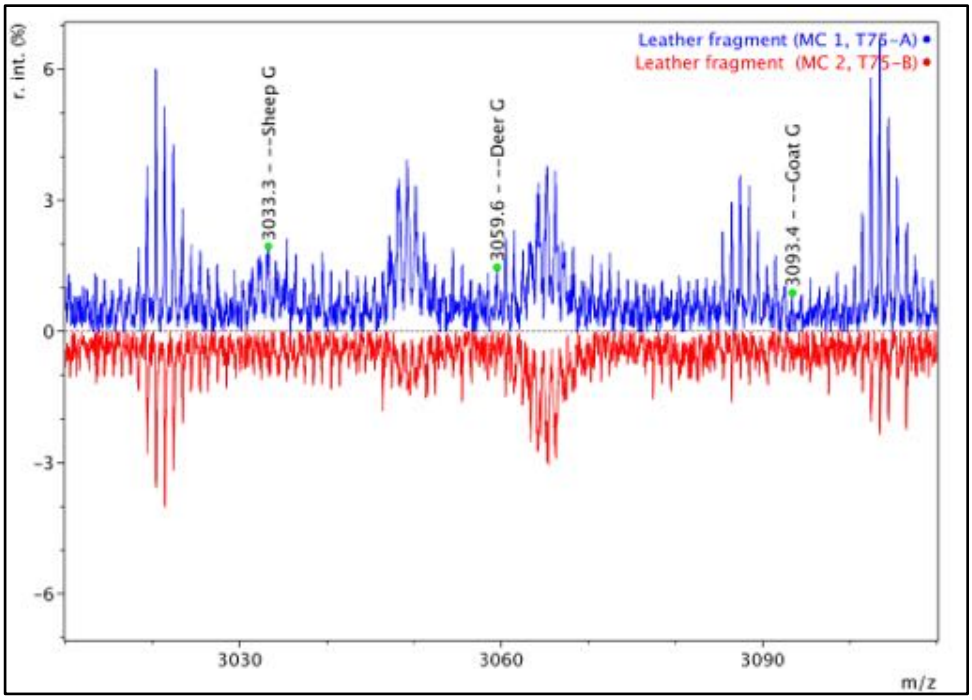


Figure 26. PMF's (partial) from two leather fragment (8945) samples illustrating the reduced quality in the high mass region where G markers are observed.

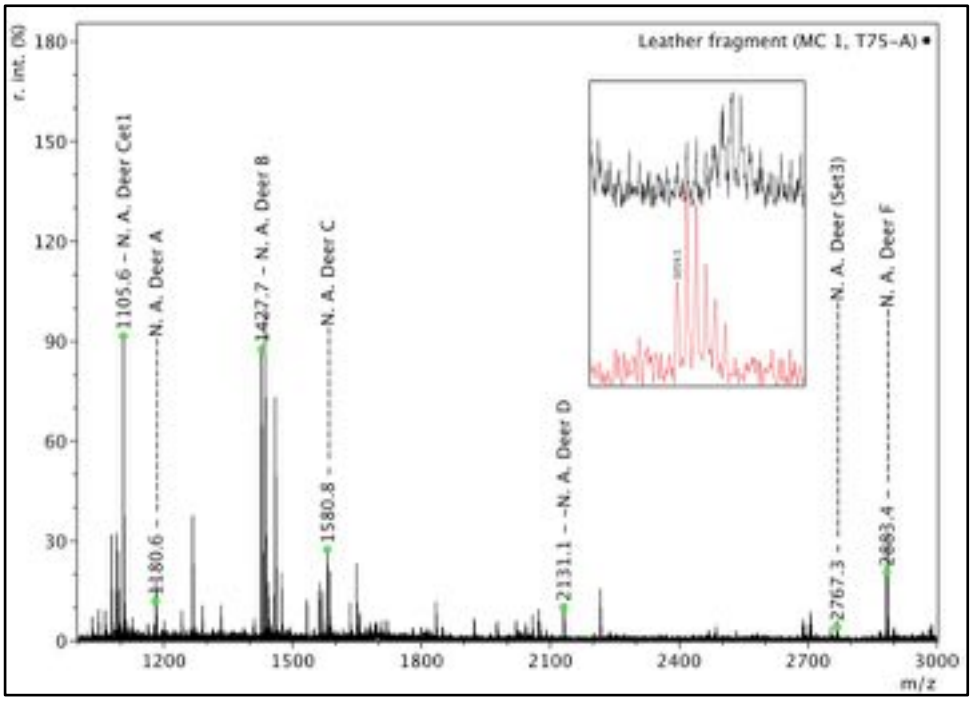


Figure 27. PMF from the leather fragment (8945) with markers for North American deer indicated. The inset shows the G marker region from the spectra of the original sample (upper, black) and the ZipTip® fraction (lower, red) confirming the G marker at 3059 Da.

Summary of results

Both sets of archaeological samples are North American deer.

3.3.4 Model of kayak with 2 harpoon head models, Peabody Museum of Archaeology and Ethnology, Museum no. 08-4-10/72929.

Background

This model kayak (figure 28) is one of twenty skin-covered models from Alaska Native groups in the Peabody Museum collection.[26] The shape of a full-sized kayak's bow and stern and the sewing technique used are unique to each Alaska Native cultural area, and the kayak models follow closely in design to the full-sized kayaks. The model kayak shown here is from the Norton Sound area (of Yup'ik manufacture), possibly St. Michael Island, Alaska, and likely dates to the late 19th century. The notches in the stern and bow and the wide cockpit characterize Norton Sound kayaks and help to determine the kayak's origin. Sven Haakanson[27], former executive director of the Alutiiq Museum, points out that models were often made as teaching tools for young people to learn techniques of sewing and frame construction. Small models did not require large amounts of precious materials. Unlike the full-sized kayaks, which were constructed by men and women together, individual men or boys made the kayak models.[28]

The museum's object record (abridged here) describes this beautifully crafted model as follows: "A one person Norton Sound style kayak model with many associated accessories. The frame appears to be constructed from red cedar and covered with light colored sealskin. In the interior are a grass mat and several tools (figure 29A), and on the deck are many assorted kayaking tools. Accessories on the deck include: two double paddles, one single blade paddle, a gutskin float (figure 29B), a line and harpoon holder, and a red painted spear with a bone/metal point attached by a cord. Across the deck are three hide straps with bone points on each side. The skin covering appears to have been sewn with sinew cords."

Samples from four locations were obtained and identified as:

- Main body skin: A – ringed seal.
- Gut float: B – walrus.
- Deck strap/tie: C – bearded seal.
- Sinew: D – beluga whale.


Figure 28. Kayak model showing sampling locations. ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 08-4-10/72929 (digital file# 75720077).

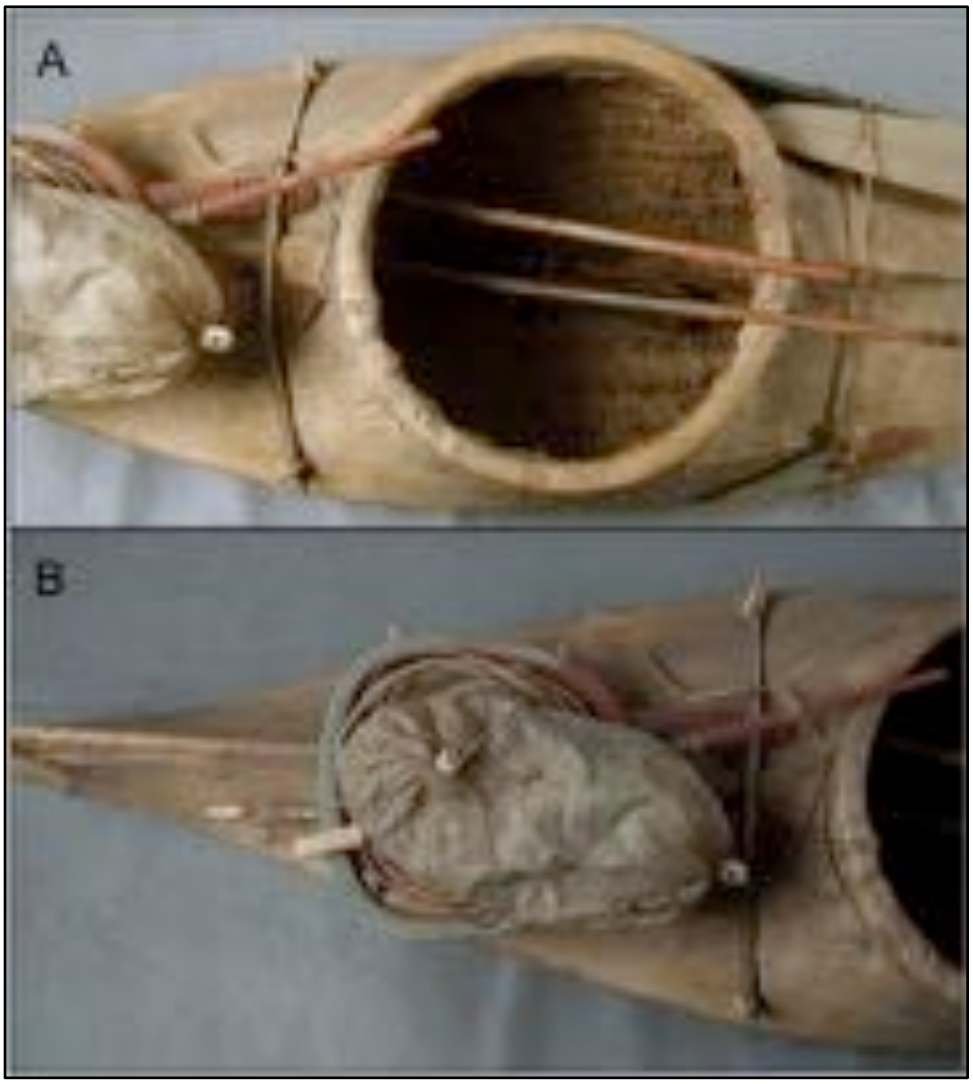


Figure 29A. Hatch detail of kayak model . ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 08-4-10/72929 (digital file# 75720079).

Figure 29B. Float and line on kayak model . ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 08-4-10/72929 (digital file# 75720078).

The materials from the kayak model were identified by PMF. Published markers (table 4, black entries) were used to identify the materials used in the kayak model. All of the kayak samples provided very high quality PMF spectra allowing the observation of all necessary markers to identify each material source to below the family level. Additional information about PMF can be found under Methods and Materials.

Samples required for PMF are micro-sized and, in general, if the sample can be seen at 30X magnification, there is sufficient material for the analysis. It is especially important with an object, such as this kayak model that is in very good condition, that samples be as small as possible to minimize impact on the object. Additional information about sampling and sample size can be found under Methods and Materials.

Analytical results

Figure 30 is the PMF from the skin of the kayak model (figure 28, location A) identifying that material as ringed seal. Ringed seal is a member of the phocini tribe of the phocidae family, the so-called true or earless seals. Ringed seal can be differentiated from all other phocini seals in the database (ribbon, spotted, gray, harbor and harp) based on the (P1) marker at 2232 Da, instead of 2216 Da. Table 11 below (a subset of table 4) contains PMF markers for all the sea mammals in the database.

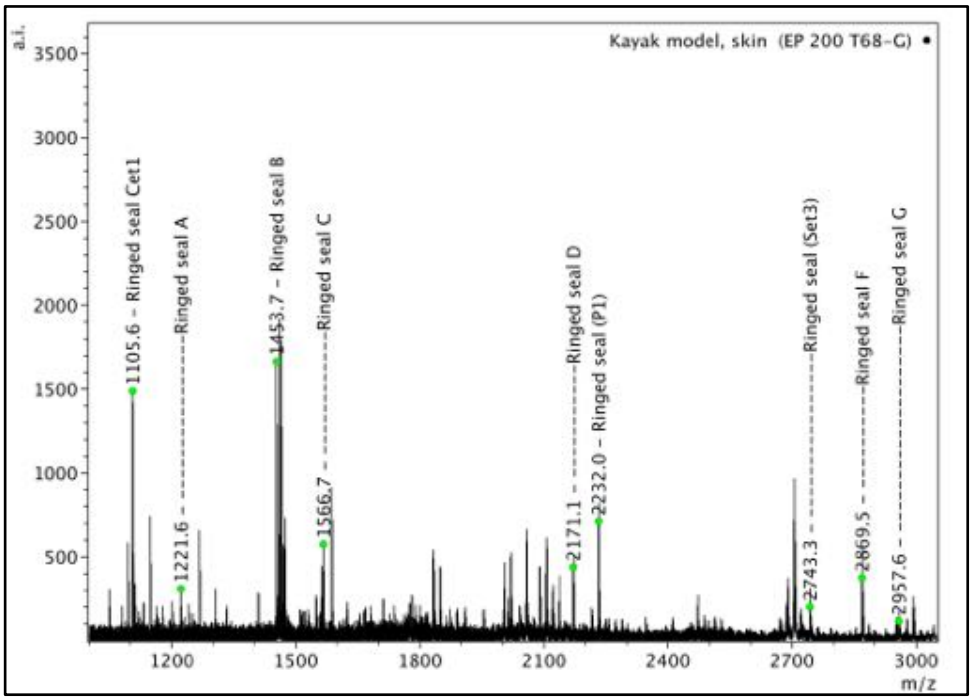


Figure 30. PMF from the kayak skin, figure 28, location A: ringed seal.

Figure 31 is the PMF from the gut float (figure 28, location B and figure 29B), which is identified as walrus. Walrus is the only surviving member of the odobenidae family of pinnipeds and can be identified uniquely among the marine mammals in the database. Walrus is readily distinguished from the closely related otariidae family, represented in the database by Northern fur seal and Steller sea lion, with the (P1), (Set3) and G markers, all of which are observed in the walrus PMF.

Figure 32 is the PMF from the deck strap (figure 28, location C), which is identified as bearded seal. Bearded seal belongs to the phocidae family (true or earless seals), as do the phocini seals in the database. Bearded seal can be differentiated from other phocidae by the Cet1 marker at 1121 Da.

Figure 33 is the PMF from the sinew stitching (figure 28, location D), which is identified as beluga whale. Beluga whale is one of two members of the monodontidae family, the other member being narwhal. Both members of monodontidae are included in the database and are readily differentiated from other cetaceans by the B marker (1443 Da) and from one another by the D marker (2089 Da *vs.* 2121 Da).

Summary of results

The kayak model's skin is ringed seal, the gut float is walrus, the deck strap is bearded seal and the sinew stitching is beluga whale.

| Reference | Cet1 | (A) | (B) | (C) | Cet2 | (D) | (P1) REF | (P2) REF | (Set3) REF | (F) | (G) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Walrus | 1105 | 1221 | 1453 | 1566 | 1652 | 2121 | 2246 | 2342 | 2731 | 2853 | 3003 |
| Northern fur sea/Steller sea lion | 1105 | 1221 | 1453 | 1566 | 1652 | 2121 | 2216 | 2342 | 2757 | 2853 | 2957 |
| Bearded seal | 1121 | 1221 | 1453 | 1566 | 1652 | 2171 | 2216 | 2332 | 2755 | 2853 | 2957 |
| Ringed seal | 1105 | 1221 | 1453 | 1566 | 1652 | 2171 | 2232 | 2346 | 2743 | 2869 | 2957 |
| Phocini seal: ribbon, spotted, grey, harbor, harp | 1105 | 1221 | 1453 | 1566 | 1652 | 2171 | 2216 | 2346 | 2743 | 2869 | 2957 |
| Hooded seal | 1105 | 1221 | 1453 | 1566 | 1652 | 2171 | 2216 | | | 2853 | 2957 |
| Cattle / Bison | 1105 | 1208 | 1427 | 1580 | | 2131 | 2199 | | 2767 | 2853 | 3033 |
| Sheep/Pronghorn | 1105 | 1196 | 1427 | 1580 | | 2131 | 2199 | | 2767 | 2883 | 3033 |
| Goat | 1105 | 1196 | 1427 | 1580 | | 2131 | 2199 | | 2767 | 2883 | 3093 |
| Musk Ox | 1105 | 1208 | 1427 | 1580 | | 2131 | 2199 | 2348 | 2769 | 2883 | 3033 |
| Elk/Red Deer/Fallow deer | 1105 | 1196 | 1427 | 1550 | | 2131 | 2199 | | 2767 | 2883 | 3033 |
| Caribou/Reindeer | 1105 | 1166 | 1427 | 1580 | | 2131 | 2199 | | 2767 | 2883 | 3093 |
| Roe Deer | 1105 | 1196 | 1427 | 1550 | | 2131 | 2199 | | 2769 | 2883 | 3059 |
| North American deer: mule, Sitka, whitetail | 1105 | 1196 | 1427 | 1580 | | 2131 | 2199 | | 2767 | 2883 | 3059 |
| Horse | 1105 | 1198 | 1427 | 1550 | 1682 | 2145 | | | | 2883 | 2999 |
| Dolphin: common, bottlenose, white-beaked, euphrosyne | 1079 | 1205 | 1453 | 1566 | 1638 | 2119 | 2225 | | 2767 | 2883 | 3023 |
| Risso's Dolphin / Pilot whale / false killer whale | 1063 | 1205 | 1453 | 1566 | 1638 | 2119 | 2225 | | 2767 | 2883 | 3023 |
| Orca / White-sided dolphin | 1079 | 1205 | 1453 | 1566 | 1652 | 2119 | 2225 | | 2767 | 2883 | 3023 |
| Porpoise | 1079 | 1205 | 1453 | 1550 | 1652 | 2119 | 2225 | | 2767 | 2883 | 3023 |
| Narwhal | 1079 | 1205 | 1443 | 1550 | 1652 | 2089 | 2225 | | 2777 | 2883 | 3051 |
| Beluga whale | 1079 | 1205 | 1443 | 1550 | 1652 | 2121 | 2225 | | 2777 | 2883 | 3051 |
| Sperm whale | 1079 | 1205 | 1453 | 1550 | 1652 | 2133 | 2225 | | 2747 | 2883 | 3039 |
| Bottlenose / Sowerby's whale | 1063 | 1205 | 1441 | 1550 | 1638 | 2091 | | | | 2883 | 3023 |
| Minke whale | 1079 | 1205 | 1441 | 1566 | 1652 | 2135 | 2225 | | 2757 | 2883 | 3023 |
| Fin Whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | | 2757 | 2883 | 3023 |
| Humpback whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | | 2777 | 2869 | 3023 |
| Blue whale | 1079 | 1205 | 1453 | 1550 | 1652 | 2105 | | | 2757 | 2883 | 3023 |
| Gray whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | | | 2899 | 3023 |
| Sei whale | 1079 | 1205 | 1441 | 1550 | 1652 | 2135 | | | | 2883 | 3023 |
| Right whale | 1079 | 1205 | 1453 | 1566 | 1682 | 2135 | 2225 | | 2789 | 2883 | 3023 |

Table 11. PMF markers for mammalian references in the database.

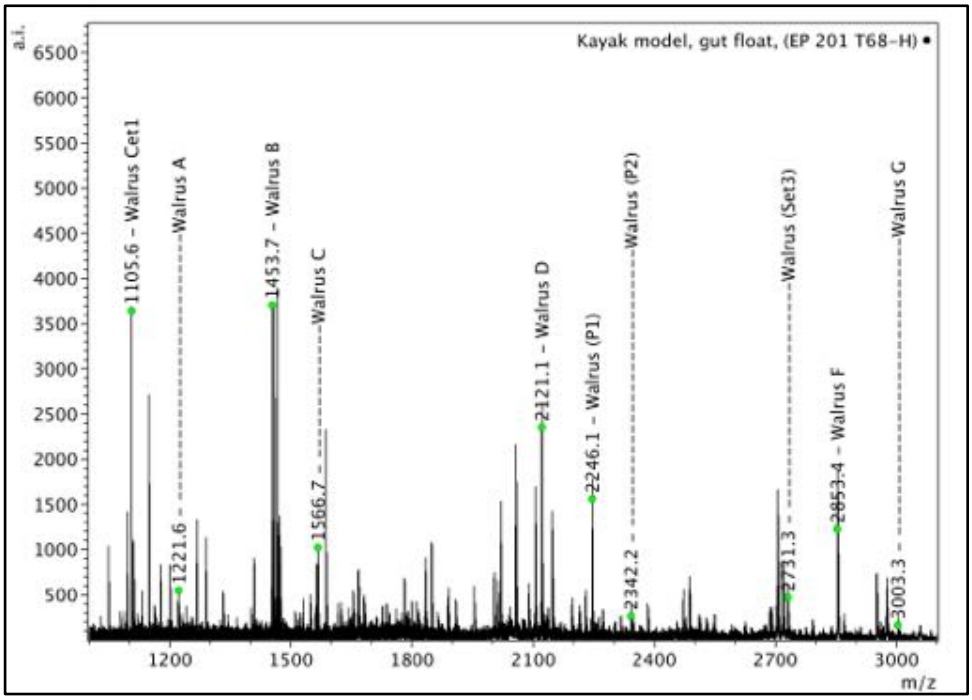


Figure 31. PMF from the gut float, figure 28, location B and figure 29B: walrus.

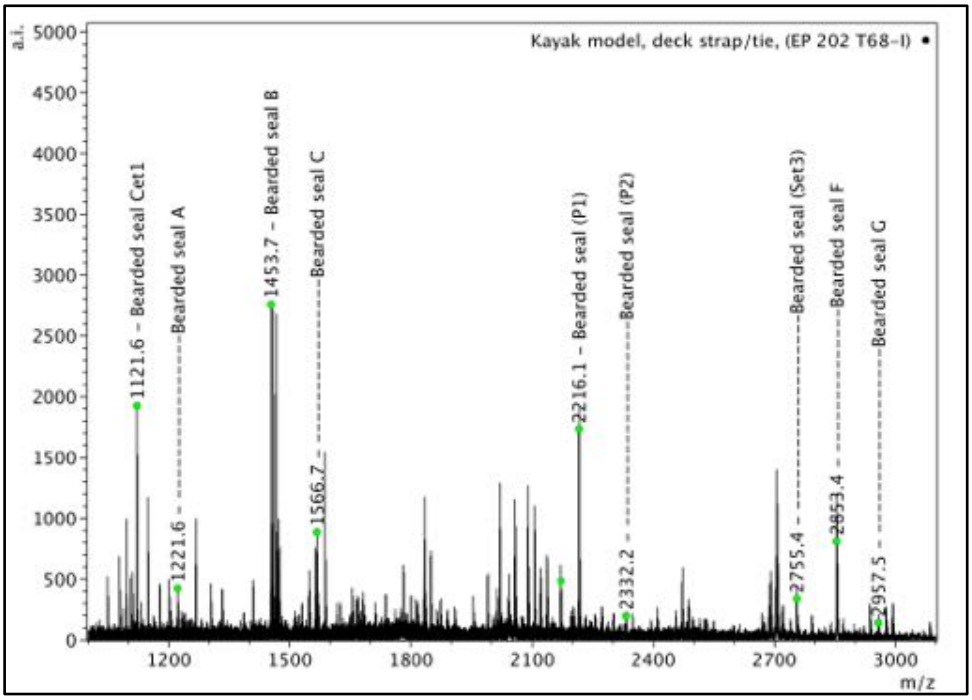


Figure 32. PMF from the deck strap, figure 28, location C: bearded seal.

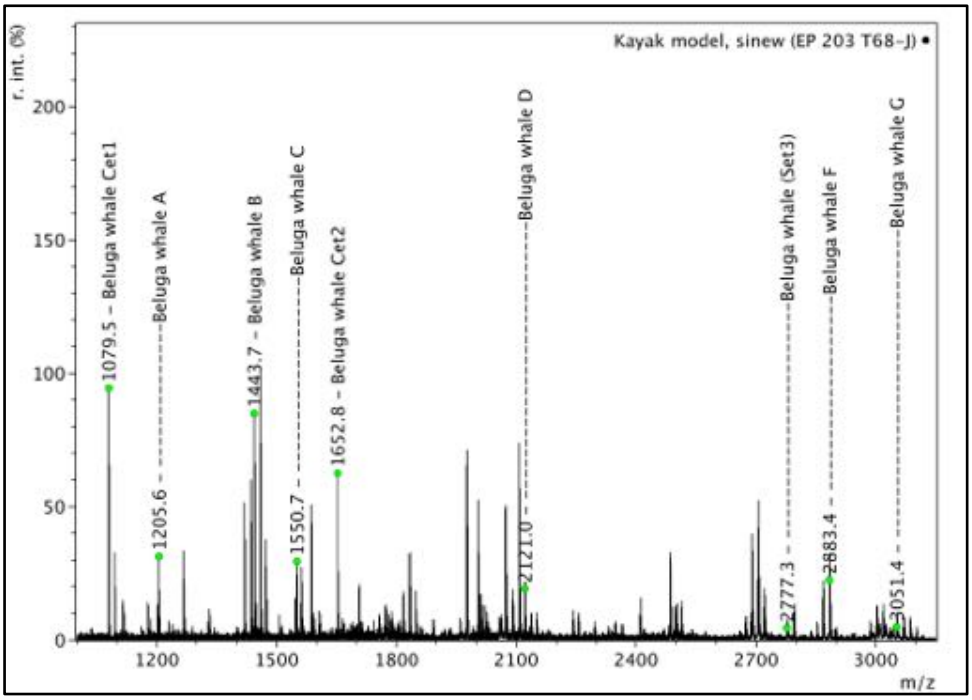


Figure 33. PMF from the sinew stitching, figure 28, location D: beluga whale.

3.3.5 Two gutskin objects

- Child's waterproof garment, Peabody Museum of Archaeology and Ethnology, Museum no. 08-8-10/73025.1.
- Skirt for kayak manhole, Peabody Museum of Archaeology and Ethnology, Museum no. 03-40-10/62814.

Background

Gutskin is a material made from mammalian inner organs and is the basis of a wide range of Alaska Native artifacts. Most of the gutskin used to manufacture artifacts is from the gastrointestinal tract including esophagus, stomach, large intestine, small intestine, and bladder. A major consideration in choosing gutskin is the fact that it is waterproof and thus is found in objects like the child's garment and kayak skirt examples shown here. Special sewing techniques have been developed by Alaskan Native workers to provide waterproof seams.

Child's waterproof garment

The child's garment (figure 34) is described in the museum object record as a "coat, intestine, small, red and blue yarn trim, and hairs at seams" and perhaps of Caribou Eskimo origin. The parka is made from strips of tan translucent mammalian intestine, sewn together using sinew. Alutiiq skin sewer Susan Malutin pointed out during an on-site consultation that the stitch is a lace stitch and that the seams are folded in a particular way for waterproofing.[29] Women from different regions in Alaska have specific ways of constructing the seams, making provenance identification possible.

Blue and red wool yarns, as well as human hair, were stitched into the seams of this small parka for the purpose of wicking away water. For dance and other ceremonial gutskin garments, there was often elaborate fur and bird skin embellishments. Susan Malutin noted that she had not seen a child's parka decorated with human hair. She speculated about the purpose of this added decoration in a garment that a child would quickly outgrow. Although waterproof seaming and stitching techniques were used, the decoration and lack of drawstrings suggested to her that this parka might have been used in a special ceremony, perhaps for a baptism.

The materials were identified as:

- Main gut material: A – bear.
- Sinew: B – caribou.

Figure 35, Sven Haakanson, former Executive Director of the Alutiiq Museum, Kodiak, Alaska, processing raw gutskin material: bear intestines.

Skirt for kayak manhole

The skirt (figure 36) is described in the museum object record as a "skirt for kayak, intestine, sinew drawstrings, tabs, hide reinforcements" and noted in the record to be from Kodiak Island, Alaska.

The materials were identified as:

- Main gutskin: A – eared seal.
- Sinew drawstring: B – humpback whale.
- Hide reinforcements: C – goat, sheep or North American deer.
- Gutskin overlay: D – eared seal.

PMF was used to identify the sources of mammalian materials used in the gutskin objects discussed here as well as materials from a wide variety of Alaskan Native artifacts, including kayaks, boots, models, etc., discussed elsewhere in this report. Until now, identification of material sources for these kinds of objects has relied on visual and/or tactile examination, and results depend greatly on the experience of the examiner and the condition of the material. In cases such as sinew and sometimes gut, where characteristic morphology needed to verify a particular source is absent, identification is nearly impossible.

Although PMF can identify mammalian materials, it cannot differentiate among the various sources of the material within the mammal, and classifying materials as intestine, esophagus, or bladder, for example, still depends on expert examination. Figure 37 compares PMF's of hide and esophagus from northern fur seal to illustrate how closely the spectra match. Only minor differences are observed between the spectra, and both contain all the PMF markers for northern fur seal. Additional information about PMF can be found under Methods and Materials.


Figure 34. Child's waterproof garment showing sampling locations. ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 08-8-10/73025.1 (digital file# 75720082).

Figure 35. Sven Haakanson cleaning bear intestines at the Port of Kodiak water dock, St. Paul Harbor, Kodiak Island, Alaska. © 2014 Jill HH Lipka, photographer.


Figure 36. Skirt for kayak manhole showing sampling locations. ©2015 President and Fellows of Harvard College, Peabody Museum of Archaeology and Ethnology, PM# 03-40-10/62814 (digital file# 75720083).

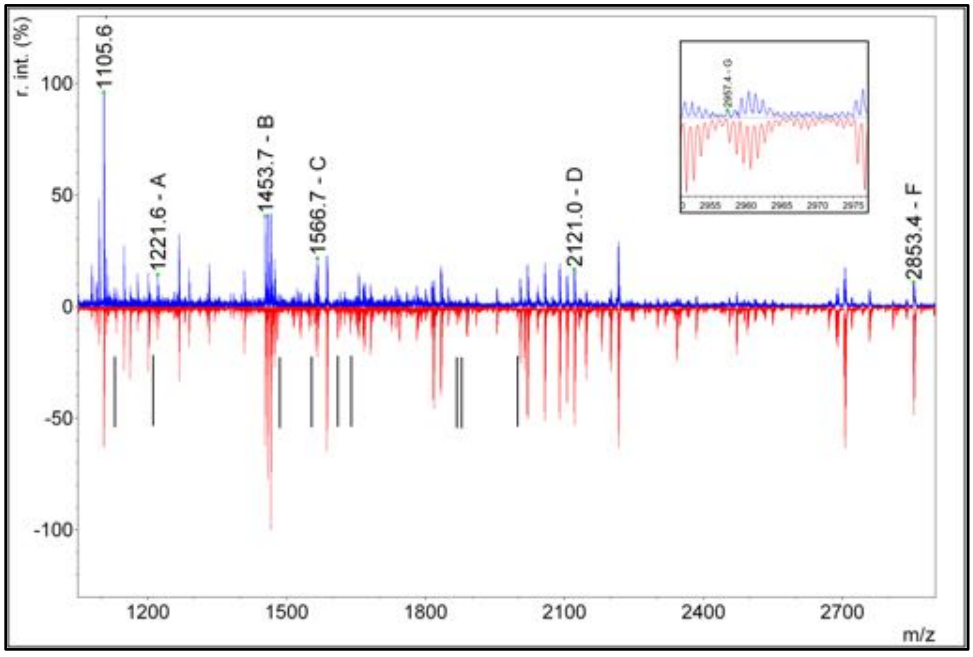


Figure 37. Comparison of PMF's from northern fur seal hide (blue, top) and esophagus (red, bottom) with markers from table 4, Methods and materials, indicated. Pointers in the lower spectrum indicate minor, additional ions observed in the esophagus sample.

Analytical results

Figure 38 is the PMF from the main gut material in the child's waterproof garment (figure 34, location A) identifying that material as bear. All bear markers are observed except G, which is not needed. Bear is readily identified among the mammals in the database by the combination of A (1233 Da) and D (2163 Da) markers. Note that PMF is unable to distinguish among members of ursidae (polar, brown and black bears).

Figure 39 is the PMF from the sinew in the child's waterproof garment (figure 34, location B) identifying that material as caribou. All caribou markers are observed except G, which is not needed. Caribou is readily identified among the mammals in the database by the A (1166 Da) marker.

Figure 40 is the PMF from the main gut material of the kayak skirt (figure 36, location A) and is the same as the PMF for the gut overlay (figure 36, location D). These materials are identified as eared seal, a marine mammal from the otariidae family. Steller sea lions and northern fur seals represent otariidae in the database, and these two mammals are not distinguishable by PMF.

Figure 41 is the PMF from the sinew used in the kayak skirt (figure 36, location B) and is identified as humpback whale. Humpback whales are members of the infraorder cetacea, marine mammals including whales, dolphins, and porpoises. PMF's of cetaceans are characterized by a Cet1 ion at either 1063 or 1079 Da and an A ion at 1205 Da (table 12, a subset of table 4). Among the 22 cetaceans in table 12, ten whales including humpback, can be identified uniquely. The shading in table 12 illustrates the successive use of markers to arrive at the identification of humpback whale. It is fortuitous that cetaceans have a high diversity of markers allowing the unique identification of many.

Figure 42 is the PMF from the hide reinforcements at the corners of the kayak skirt (figure 36, location C). The observed combination of A, B, C, D, (Set3) and F ions is identical for goat, sheep and North American deer, and the G marker is needed to differentiate among these. The G marker (3033, 3059 or 3093 Da), however, could not be confidently identified after several attempts at ZipTip® fractionation, possibly because of the degraded condition of the material. See Methods and Materials for more information on ZipTip® fractionation.

Summary of results

For the child's waterproof garment, the main gut material is bear and the sinew is caribou. For the kayak skirt, the main gut material and gut overlay are eared seal, the sinew is humpback whale, and the hide reinforcements are either goat, sheep or North American deer.

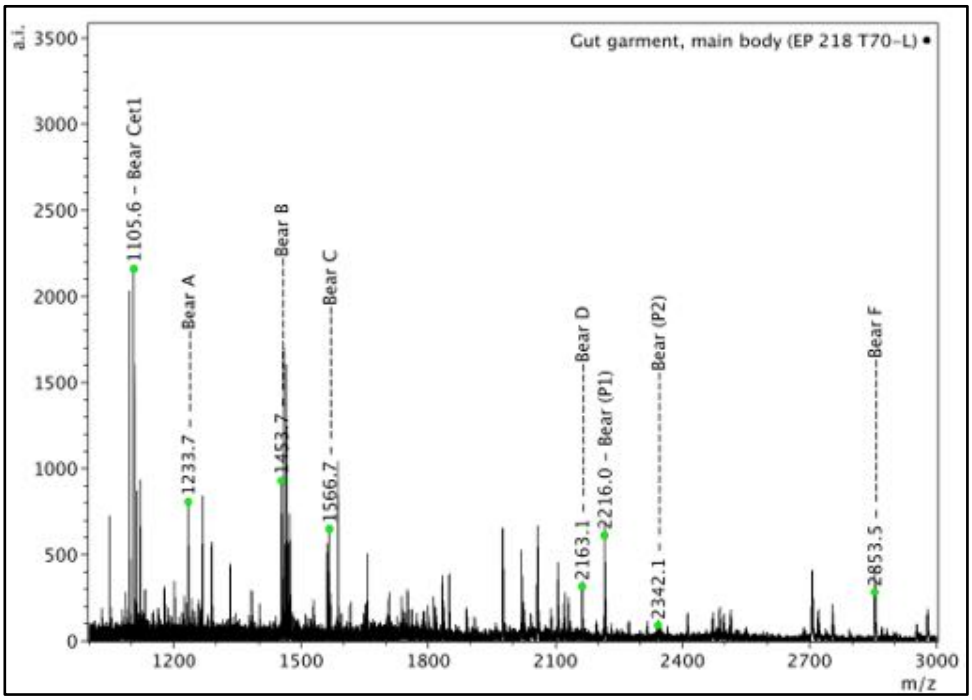


Figure 38. PMF from the garment's main gut material in figure 34, location A: bear.

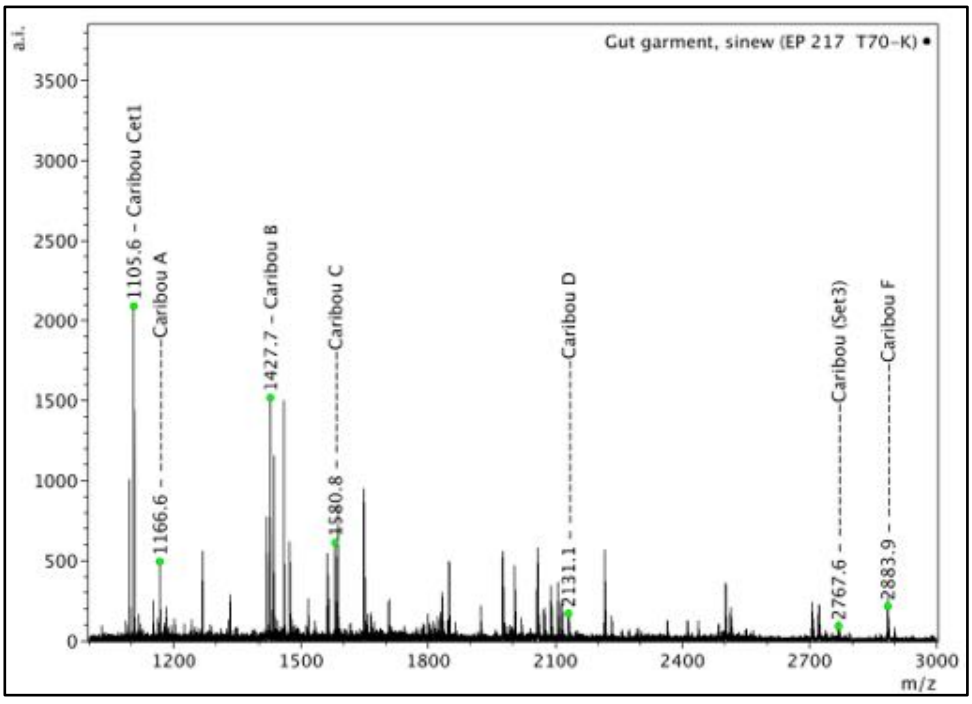


Figure 39. PMF from the garment's sinew in figure 34, location B: caribou.

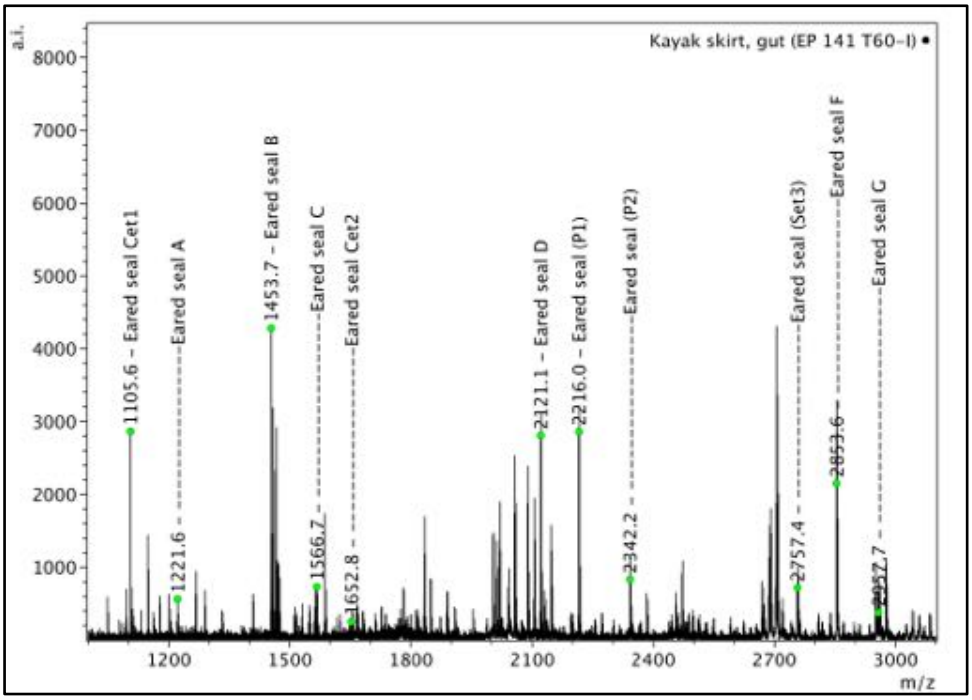


Figure 40. PMF from the skirt's main gut material and gut overlay in figure 36, locations A and D: eared seal.

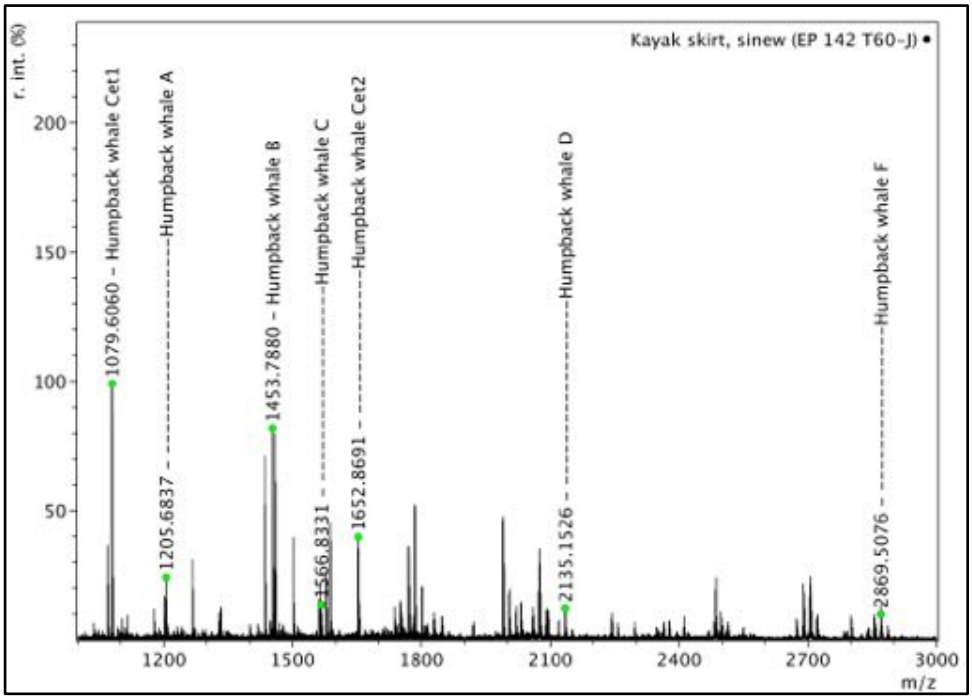


Figure 41. PMF from the skirt's sinew in figure 36, location B: humpback whale.

| | Cet1 | (A) | (B) | (C) | Cet2 | (D) | (P1) REF | (F) | (G) |
|---|---|---|---|---|---|---|---|---|---|
| Common / Bottlenose / White-beaked / Euphrosyne dolphin | 1079 | 1205 | 1453 | 1566 | 1638 | 2119 | 2225 | 2883 | 3023 |
| Risso's Dolphin / Pilot whale / false killer whale | 1063 | 1205 | 1453 | 1566 | 1638 | 2119 | 2225 | 2883 | 3023 |
| Orca / White-sided dolphin | 1079 | 1205 | 1453 | 1566 | 1652 | 2119 | 2225 | 2883 | 3023 |
| Porpoise | 1079 | 1205 | 1453 | 1550 | 1652 | 2119 | 2225 | 2883 | 3023 |
| * Narwhal | 1079 | 1205 | 1443 | 1550 | 1652 | 2089 | 2225 | 2883 | 3051 |
| * Beluga whale | 1079 | 1205 | 1443 | 1550 | 1652 | 2121 | 2225 | 2883 | 3051 |
| * Sperm whale | 1079 | 1205 | 1453 | 1550 | 1652 | 2133 | 2225 | 2883 | 3039 |
| Bottlenose / Sowerby's whale | 1063 | 1205 | 1441 | 1550 | 1638 | 2091 | | 2883 | 3023 |
| * Minke whale | 1079 | 1205 | 1441 | 1566 | 1652 | 2135 | 2225 | 2883 | 3023 |
| * Fin Whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | 2883 | 3023 |
| * Humpback whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | 2869 | 3023 |
| * Blue whale | 1079 | 1205 | 1453 | 1550 | 1652 | 2105 | | 2883 | 3023 |
| * Gray whale | 1079 | 1205 | 1453 | 1566 | 1652 | 2135 | 2225 | 2899 | 3023 |
| * Sei whale | 1079 | 1205 | 1441 | 1550 | 1652 | 2135 | | 2883 | 3023 |
| * Right whale | 1079 | 1205 | 1453 | 1566 | 1682 | 2135 | 2225 | 2883 | 3023 |

Table 12. PMF markers used to identify cetaceans. The shading illustrates the process of successively eliminating entries to arrive at humpback whale as the source of the sinew.

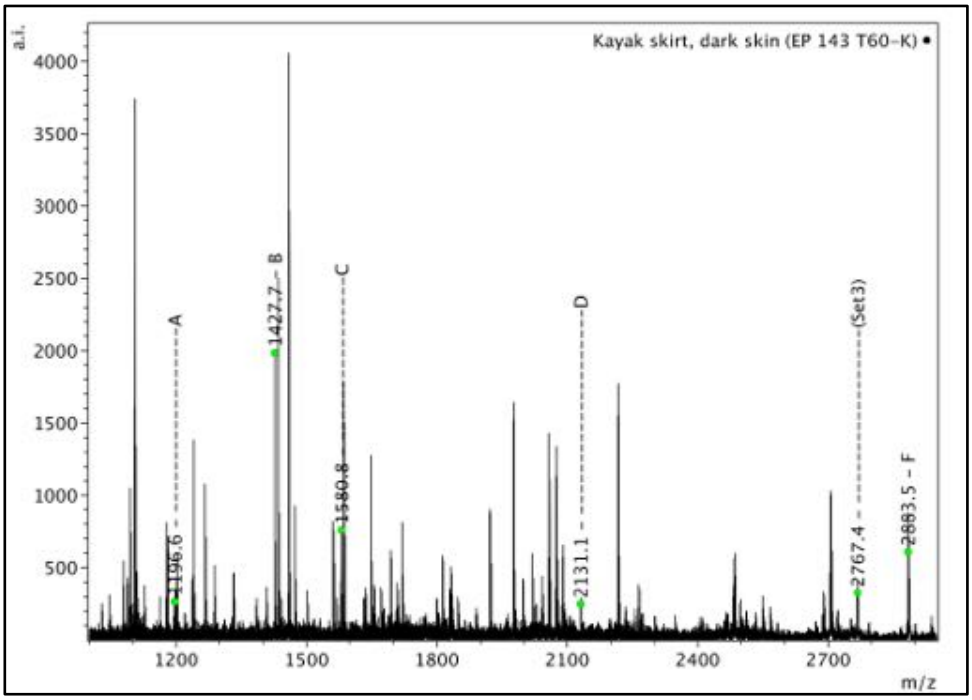


Figure 42. PMF from the skirt's edge reinforcement, figure 36, location C: goat, sheep, or North American deer.

4. Conclusions

PMF was successfully demonstrated. Through this NPS grant-funded research, the application of PMF to the study of collagen-based materials in the Peabody Museum's collection has been successfully demonstrated. The project's focus was on skin-constructed objects from Alaskan coastal areas, the Northwest Coast, and the High Plains, but the focus could equally well have been on other geographical areas as the method would have been the same. The results of this study are being used to reach a more complete understanding of the many objects that were sampled, to corroborate and expand traditional knowledge, and to update existing, often limited museum documentation. This is the first time that PMF has been used in a large-scale survey of materials in any museum collection.

Multiple researchers were trained. Significantly, over the course of the work, multiple researchers, most of whom were initially inexperienced with the technique, performed analyses independently and successfully after only a few days instruction. Typically, samples were analyzed in batches of up to 25 and results were available in two to three days. Quick turnaround time for large numbers of samples opens the possibility of extensive, collaborative studies of objects of similar type and provenance across collections in different institutions.

Cultural stakeholders will benefit. Ellen Promise, the first Peabody researcher involved with PMF analysis, summarized her work on the Alaska Native kayak study at ICOM-CC, Melbourne[30] this way: "The PMF technique has allowed a considerable amount of data about diverse materials to be collected within a relatively brief time frame. Confirmed species identification will allow researchers to better understand the availability of specific materials in a given region and, in some cases, help in sourcing an object of unknown provenance. Artisans and cultural groups can also use the information in their efforts to better understand and sustain their native heritage."

New reference materials were documented. An important part of this project was the analysis of approximately 200 new reference materials and the discovery of provisional markers for several new mammalian families, which were immediately useful for data interpretation.

PMF awareness was raised. By far, however, the most important outcome of our work is the raising of an awareness of the potential of this relatively simple technique to curators, conservators, and cultural stakeholders through the wide dissemination of our results. Our goal is that museum professionals see PMF as a powerful, stand-alone method in addition to being an excellent supplement to existing methods used for materials studies. This is an especially important consideration for historic materials, such as gut and tendon, which are generally difficult to assess with visual/tactile techniques, and archaeological materials, which may have lost all identifiable features.

In-house questions were answered. In this project's final months, the Pueblo of Zuni, during the course of a tribal consultation, requested assistance with materials identification for a bow and arrows with sinew in the Peabody Museum's collection. They asked that analysis be undertaken to determine whether the sinew was from deer or pronghorn antelope. PMF identified North American deer as the source of the sinew, an

important piece of information to the representatives from the Pueblo of Zuni toward their cultural understanding of these early-20th century objects. The benefits of PMF analysis to the study of previously unknown sinew, gut, and processed skin material are obvious; existing methods of analysis would have failed. This example clearly illustrates the significant value of PMF as a tool for material studies whether used alone or, more importantly, used in concert with other techniques to provide a clear, accurate picture of material cultural heritage.

5. Recommendations for further work

Establish a focal point for future research and applications. The results of this project validate the use of PMF for materials' study in objects of cultural heritage. Going forward, it will be important that a focal point be established to act as a technical resource for researchers and institutions desiring to develop their own PMF capability. The same resource could also take the lead in implementing the additional recommendations below.

Expand reference database. An important part of this project was the analysis of approximately 200 new reference materials and the discovery of provisional markers for several new mammalian families, which were immediately useful for interpreting our data. During the course of this work, the need to expand existing databases to include additional material sources, such as fish and bird skins, became obvious and is an important consideration for future work.

Include keratin-based materials. Our work considered only collagen-based materials, which covers a large number of objects but neglects another large group: those containing keratin-based materials such as fur, feathers, horn, and hoofs. It is a logical and necessary extension of the PMF work to expand the database and methods to include this important class of materials.

Develop automated search. Finally, as the number of references continues to grow, it will become increasingly important to be able to search spectra automatically, as is done with FTIR and Raman spectroscopies. This capability will increase accuracy and ease of identification and facilitate the routine use of PMF in a museum environment.

6. Acknowledgements

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Judy Jungels, conservator at the Peabody Museum, Harvard University

Sven Haakanson, Associate Professor and Curator, University of Washington and Burke Museum, Seattle WA (formerly Executive Director at the Alutiiq Museum, Kodiak, Alaska).

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Renee Robinson of the Harvard FAS Division of Science's Mass Spectrometry and Proteomics Resource Laboratory

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Corina Rogge, Museum of Fine Arts, Houston, TX

Joseph Carvalho, Joe's Taxidermy, Norton, MA

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8. Appendix/Dissemination of Project Methodology and Results

Dissemination of the project activities and the training of young professionals in the use of PMF were core components of the NCPTT grant project, P13AP00078. The goals were to disseminate methods and results of the project's research as widely as possible and to receive feedback from colleagues to better understand future needs for application of this biotechnology technique for purposes of enhancing understanding and preservation of cultural heritage collections.

The following forums served to meet these goals:

Project Website

To promote PMF as a routine method for the identification of proteinaceous materials found in museums, the project team developed a website which contains all pertinent information about the project, including procedures, updates, case studies, and reference sources. The website was organized through the FAS Division of Science-Small Molecule Mass Spectrometry Facility supported by the Projects at Harvard website. Website address: http://projects.iq.harvard.edu/pmfcm

Workshop on PMF

The website was also the forum for participant registration and sharing of information on the PMF workshop. The event took place on May 8, 2014 at Harvard University in collaboration with the Peabody Museum of Archaeology and Ethnology, Harvard FAS Division of Science-Small Molecule Mass Spectrometry Facility, and the Harvard University Art Museums with funding from the NCPTT grant. The workshop entitled "Identifying Collagen-Based Materials in Cultural Objects," was publicized through the American Institute for Conservation (conservation-distlist), on the Harvard-Peabody-NCPTT project's website (see above), through the New England Conservation Association membership, and by individual email notifications.

Organization of the workshop

The program featured an introduction and four in-depth presentations by the project team with one contributed by Ellen Promise, a 2012 postgraduate conservation fellow at the Peabody Museum who had applied PMF to skin-covered kayaks as part of a 2010 Save America's Treasures grant project. These presentations provided participants with background information and thorough review of the procedures associated with the technique, as well as pertinent results illustrated through several case studies. The presentations were followed by a question and answer period and a discussion of future directions. The latter part of the program allowed participants the option to take tours with project team members to the Peabody Museum conservation lab and to the Small Molecule Mass Spectrometry Facility.

The tours provided the participants an opportunity to observe first hand practical demonstrations of the extraction/digestion protocol and sample analysis on the Bruker MALDI-TOF-TOF instrument as well as to study and discuss the analytical results in physical association with several Alaska Native skin and gut objects from the Peabody Museum's collection.

Participants

Of the 50 participants, one-half were from the Harvard museums and libraries, and the other traveled to the Harvard-Cambridge campus from eleven different museums or university laboratories from across the New England states including Pennsylvania. There were several individuals from independent art conservation businesses and two members of the Wampanoag Tribe of Gay Head-Aquinnah. Workshop participants were given handouts from the website, http://projects.iq.harvard.edu/pmfcm as well as a three-page 'Procedures for making peptide mass fingerprints to identify proteins,' along with a list of chemicals, equipment, consumables, and suppliers. These materials were subsequently uploaded to the website for future access.

The workshop generated positive feedback and the project team has since received numerous inquiries about setting up PMF at other institutions. Interested parties were sent detailed instructions on the PMF procedure. The project research associate also created a "PMF Startup Package" which includes instructions on data processing using mMass and practice data interpretation files. These files were transmitted to individuals who wanted further assistance in applying the procedure to objects from their own collections. The project scientist and project research associate addressed inquiries *via* email related to assistance with initial setup.

Professional and Informal Presentations and Published Articles

Other beneficial forums for sharing more widely on the project's activities and on PMF, included a variety of presentations, published articles and short written communications.

*July 27-August 1 2014 Gordon Research Conference.* Summary information generated by the PMF workshop was provided to Matthew Collins (of the University of York, United Kingdom) for presentation "Collagen-Based Materials: Characterization and Preservation" delivered at the "Scientific Methods in Cultural Heritage Research" session of the Gordon Research Conference, Newry, Maine. Attendees to this conference included scientists and conservators from various locations in the United States.

*Spring 2014. ICOM-CC Leather Conservation Group Newsletter*. A short written update on progress on the NCPTT research project and PMF application of skin, sinew and gut on Alaska Native, Northwest Coast and High Plains objects was submitted and accepted.

*May 2014: American Institute for Conservation Annual Meeting.* A paper was delivered at the Objects Specialty Group session of the meeting held in San Francisco. A component of the paper described the importance of the ongoing NCPTT project on the PMF analysis of skin, sinew and inner membranes on Alaska Native objects. See appended article entitled: "Collaborative study and preservation of coastal Alaskan Native material culture with museum staff, Alutiiq scholars and artists, university students and the visiting public," by T. Rose Holdcraft, Sven Haakanson, Ellen Promise, Judy Jungels, Fran Ritchie, and Patricia Capone.

*Spring 2014. Collections: A Journal for Museum and Archives Professionals*. An article written by Judy Jungels and T. Rose Holdcraft entitled 'Study and Treatment of Coastal Alaskan Native Kayak Models," included PMF analysis of 12 models.

*November 2014. ICOM-CC 17th Triennial Conference, Melbourne*. Presentation and preprint titled "Identifying collagen-based materials: A cross-cultural collaboration" included initial analyses of the Alutiiq kayaks and emphasized the collaboration between the Peabody Museum and the Alutiiq consultants that led to NCPTT funding.

*June 2015: Alutiiq Museum, Kodiak Island*. A presentation on the 2011-2014 Save America's Treasures kayak study and conservation project included information on the continuing work identifying gut and skin on Alaska Native and specifically Alutiiq cultural heritage objects under the NCPTT grant project, in discussion with native skin sewers, kayak makers, representatives of the Natives of Kodiak, Inc., and museum professionals.

*Fall 2015: ICOM-CC Newsletter of the Objects from Indigenous and World Cultures Working Group.* Update on the NCPTT project featuring case studies on peptide mass fingerprinting (forthcoming).

Ongoing Inquiries about PMF

In response to the workshop and ongoing exchanges with participants and through subsequent presentations and written updates, the project team has received to date more than 10 inquiries regarding the PMF method. We have supplied detailed instructions for sampling and sample processing, as well as reference materials to verify their results, to several groups, including the Weissman Preservation Center at Harvard and Centre de Recherche sur la Conservation des Collections CRCC, Muséum National d'Histoire Naturelle, Paris. During the preparation of this final report, we had indications of interest in duplicating our methods from the Burke Museum of Anthropology, University of Washington and from colleagues in Stuttgart and University of Pennsylvania.

Dissemination through professional presentations

*International Council of Museums-Conservation Committee 17th Triennial Meeting, September 2014*

Selected images from the presentation featuring PMF work in procecss with NCPTT grant funding.

Table of results (partial):

- 230 samples
- 50 approved objects
- 63% previously "unknown" identified to family or species level
- 13% erroneously identified were corrected
- 13% more completely identified

| Object | Sampling | Museum ID | PMF ID |
|---|---|---|---|
| Gutskin bag (2103) | gut | seal | eared seal |
| Gutskin bag (2103) | painted stripe | seal | seal (Phocidae/phocini) |
| Gutskin bag (2103) | sinew | unknown | caribou |
| Gutskin bag (48414) | gut | walrus or sea lion | eared seal |
| Gutskin bag (48414) | black border | walrus or sea lion | bear |
| Gutskin bag (48414) | sinew | unknown | caribou |
| Gutskin bag (76018) | red painted | bear | eared seal |
| Gutskin bag (76018) | gut | bear | eared seal |
| Gutskin bag (76018) | border sinew | unknown | eared seal |
| Gutskin bag (76018) | embroidery | unknown | dog/wolf |
| Gutskin bag (48414.1) | gut | unknown | bear |
| Gutskin bag (48414.1) | red and green | unknown | bear |
| Gutskin bag (48414.1) | sinew | unknown | caribou |
| Gutskin cap (48415) | gut | seal | eared seal |
| Gutskin cap (48415) | painted border | unknown | seal (Phocidae/phocini) |
| Gutskin cap (48415) | sinew | unknown | caribou |
| Spear with pouch and | pouch | sea lion | eared seal |
| Spear with pouch and | sinew | whale | caribou |
| Gutskin coat (56749) | gut | seal | eared seal |
| Gutskin coat (56749) | black overlay | unknown | eared seal |
| Gutskin coat (56749) | sinew | unknown | eared seal |
| Kayak model (1203) | skin | unknown | seal (Phocidae/phocini) |
| Kayak model (1203) | sinew | unknown | Right whale |
| Kayak model (1204) | skin | sea lion | eared seal |
| Kayak model (1204) | sinew | unknown | Right whale |
| Kayak model (11255) | skin | unknown | eared seal |
| Kayak model (11255) | gut | unknown | eared seal |

## Jan 2014 – June 2014 (NCPTT Grant)

- 239 samples from 54 objects
- 21 different species
- ~ 35 unknowns: likely avian and fish
- 45+ new reference samples (plans for ~50 more)

## Future plans

- Extend to non-mammalian collagen sources
- Map regional materials usages
- Develop searchable, expanded databases
- Extend analyses to keratin-based materials

## Contacts

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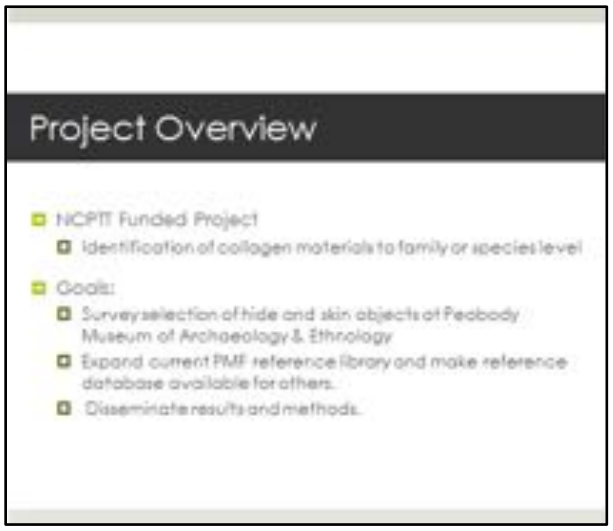
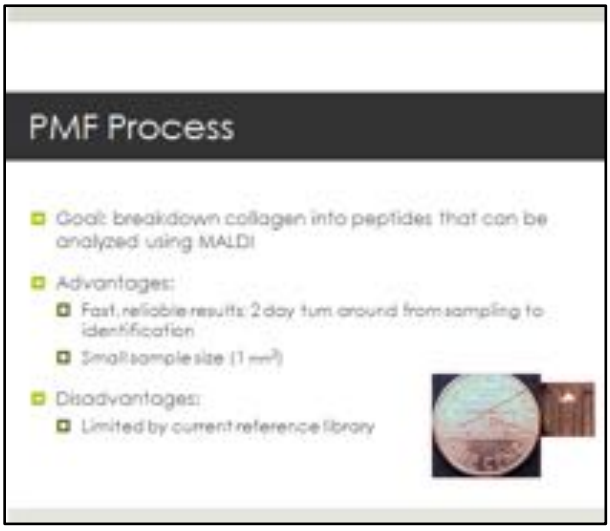
- Dan Kirby – dp.kirby@verizon.net

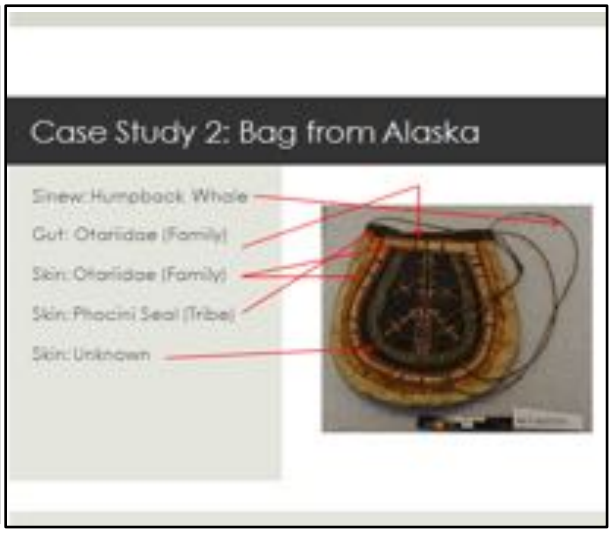
## Project website

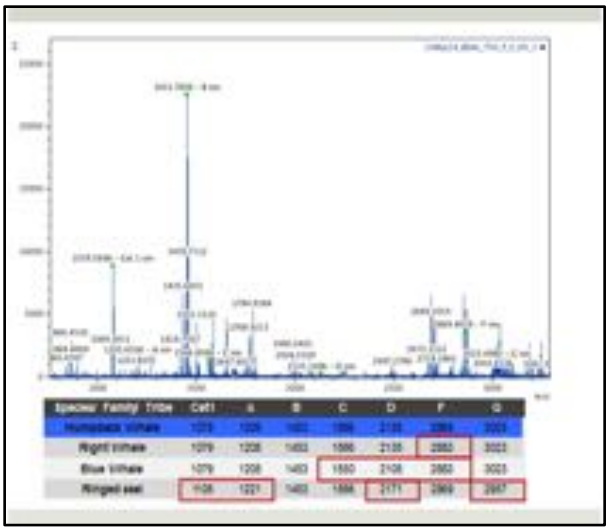
- http://projects.iq.harvard.edu/pmfcm

The following six images were shared at the May 2014 Workshop at Harvard University on PMF and case studies and then made available to the July 27-August 1, 2014 Gordon Research Conference session on "Collagen-Based Materials: Characterization and Preservation."

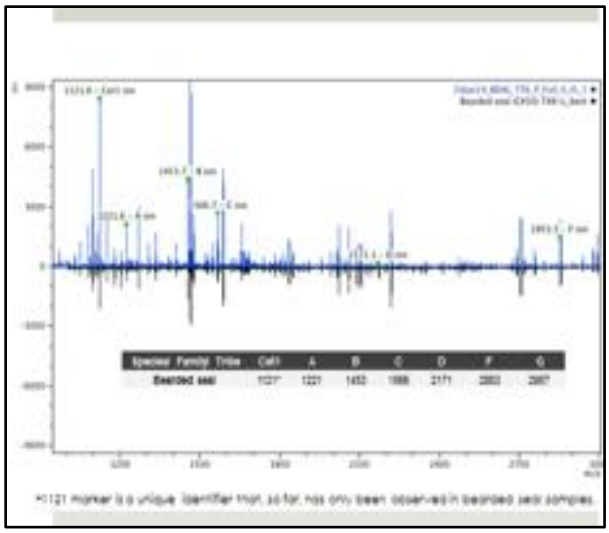





| Species/ Family/ Tribe | Cat1 | A | B | C | D | F | G |
|---|---|---|---|---|---|---|---|
| Humpback Whale | 1079 | 1208 | 1453 | 1886 | 2135 | 2862 | 3023 |
| Right Whale | 1079 | 1208 | 1453 | 1886 | 2135 | 2862 | 3023 |
| Blue Whale | 1079 | 1208 | 1453 | 1886 | 2135 | 2862 | 3023 |
| Ringed seal | 1126 | 1221 | 1453 | 1886 | 2171 | 2869 | 2967 |



| Species/ Family/ Tribe | Cat1 | A | B | C | D | F | G |
|---|---|---|---|---|---|---|---|
| Bearded seal | 1127 | 1221 | 1453 | 1886 | 2171 | 2862 | 2967 |

*1121 marker is a unique identifier that, so far, has only been observed in bearded seal samples.