Animal Bone Specimens Preparation Method

Takeshi Yamazaki
Environmental Archaeology Section
Nara National Research Institute for Cultural Properties

This text was prepared for the "Introduction to Environmental Archaeology" given in the "Asia-Pacific Region Cultural Heritage Preservation Training Course 2010 (Group Training): Study and Preservation of Archaeological Sites" conducted by the Asia-Pacific Cultural Centre for UNESCO (ACCU). Maintenance of extant specimens is the most important theme for implementing environmental archaeology (particularly bioarchaeology). This text contains a description of how to prepare extant animal bone specimens used in zooarchaeology.

1. Extant specimens

(1) Importance of comparative specimens in environmental archaeology

Identification is accomplished by analysis of plant/animal remains or human skeletal remains. Identification determines what part of the taxonomic group (family, genus, and species) the archaeological materials belong. Identification requires expertise in plants and animals and human skeletal structure.

Identification is accomplished by comparing with extant specimens. Identification of human skeletal remains sometimes involves comparison with a model skeleton. Providing photographs, drawings and morphological features serve as a reference, but excavated materials have often significantly deteriorated and must ultimately be identified by comparing with an actual specimen. In order to enhance the precision of identification, archaeological materials should be compared with extant specimens; sufficient quality and quantity of extant specimens are also required.

Extant specimens serve as evidence to support the basis of identification. Those used for comparison must be open to public inspection to ensure reproducibility of identification results. If no extant specimens are immediately available, specimens kept at museums or laboratories can be used effectively.

(2) Extant animal bone specimens in zooarchaeology

Identification in zooarchaeology involves identifying to which part of the animal taxonomic group existing animal remains (shells, animal bones fragments, teeth, horns, etc.) excavated from the site belong. Identification is a fundamental task of zooarchaeology, and involves comparing morphological features with those of extant specimens. Enhancing precision of identification requires sufficient bone specimens of extant animals.

Some museums of natural history, universities and research centers collect and administer extant animal specimens. The specimens however may not be suitable for zooarchaeology involving different research backgrounds and objectives. Fish specimens for instance are usually not kept as skeletal specimens, but are rather kept immersed in liquid. Because the skeleton cannot be observed, such specimens are not suited to identification for zooarchaeology. In the case of mammals, oftentimes only the skulls are kept as specimens. Animal remains are often unearthed in the form of an entire skeleton, rather than just a skull. Specimens for zooarchaeology consequently require the entire skeleton of extant animals to be collected, depending on the archaeological objective.

2. Preparation of extant animal bone specimens

(1) Significance of preparing extant animal bone specimens

Many skeletal diagrams and photographs that are helpful for identifying animal remains have been published. Strict identification however requires extant animal bone specimens rather than diagrams or photographs. In zooarchaeology, disconnected animal bone specimens that are not assembled are used in order to observe the particular surface. You cannot however get a good understanding of anatomical location using disconnected bone specimens kept by various institutions alone. You must therefore get an understanding of the relative positioning of all skeletal regions by preparing bone specimens.

The objective of zooarchaeology is to study animal resource use at the time from animal remains for which only hard tissue such as bones and teeth remain. Specimen preparation provides a precious opportunity to gain anatomical knowledge of bones, muscles, tendons, nerves, etc.

(2) Obtaining animals

It is first necessary to obtain animals for specimens. When doing so, laws, regulations and ordinances related to capture of wild animals must be observed and ethical issues concerning collection of live specimens must be considered. In Japan, guidelines for treatment and handling of specimens drawn up by academic societies related to animals can be accessed on their websites. Be sure to browse the guidelines. (Examples include The Mammalogical Society of Japan and The Ichthyological Society of Japan.)

Obtaining fish and shellfish: Methods of obtaining fish and shellfish include (1) collecting them yourself and (2) purchasing from dealers such as fish shops. If (1) collecting them yourself, you can reliably record data that belongs to the specimen such as that place where the fish was captured (locality). When collecting fish and shellfish you must however properly take fishing rights into account. Even if you are collecting fish or shellfish that are not applicable to fishing rights, you should be careful not to do anything that may invite misunderstanding. If (2) purchasing from dealers, be careful not to provide the product name (nominal designation). The nominal designation of fish may contain the name of a region or growth stage, which may differ from the standard Japanese name used in biology. The product name (nominal designation) must not be taken on faith; you must properly perform identification. The fish that can be purchased from a dealer are

also limited to types that have commercial value. It is therefore effective to get fisherman on docks of fishing ports to let you take the fish and shellfish culled from the catch to be discarded. Beached shells that can be collected on the seashore can also serve as specimens for analyzing shellfish remains or shellfish products.

Obtaining birds and mammals: Methods of obtaining birds and mammals include (1) capturing animals for study, (2) obtaining animals killed by hunting/extermination, (3) obtaining animals killed by accidents and (4) donation. (1) Capturing wild animals for study not only requires skill and experience, but also involves permission for trapping. Cooperation of an expert may be required. Collection of birds and mammals is currently limited by wildlife protection laws. (2) Obtaining animals killed by hunting/extermination involves using game animals or harmful animal that have been trapped for extermination. Wild boars (*Sus scrofa*) and Japanese deer (*Cervus nippon*), which are often unearthed from sites, can probably be obtained by this method. (3) Obtaining animals killed by accidents involves collecting roadkill. Mammals such as raccoon dogs and foxes are often hit by cars and trucks. Because they are roadkill, the animals are often in poor condition but can effectively serve as comparative specimens for species identification. Carcasses that wash up on shore (stranded animals) provide a precious opportunity to obtain marine mammals to use as specimens. (4) Donations involve using carcasses of dead animals donated by zoos or aquariums as specimens.

Temporary storage: If an animal or animals obtained cannot be prepared as a specimen immediately, it should be frozen for temporary storage. Small animals can be preserved in ethanol (ethyl alcohol). In the case of prolonged storage, you should keep in mind the fact that ethanol evaporates. Formalin is also available as a preservative solution, but is not recommended due to the fact that if dissolves soft tissue, is difficult to remove, and may affect bone measurement values due to formalin fixing. For temporary storage, be sure to include a label with data about how the specimen was obtained. Use water-resistant paper so the label doesn't tear or become illegible.

(3) Data belonging to specimen

Data belonging to the specimen should be preserved along with the specimen for posterity. The data will be lost if not recorded when collecting the specimen, so be sure to record whenever possible. Specific examples of basic data include date the specimen was collected, who it was collected by, where it was collected, name of species, scientific name, sex, external measurement values and method by which it was collected (or obtained).

Identification: If the species of the specimen is incorrectly recorded, animal remains unearthed at the site to be identified using the specimen will be incorrectly identified. Specimen identification therefore requires maximum care. Literature concerning Japanese mammals is available by searching Jefferson, T.A., et al (1999), Abe (2000), Abe supervision (2008), and Japanese birds by searching Morioka Edition (2003), and Japanese fish by searching Nakabo Edition (2000).

In the case of actual identification with Japanese fish as an example, after establishing an approximate target for family and genus by using several guides to fish with lots of color photographs, identify species by categorical traits of "Fishes of Japan with pictorial keys to the species, 2nd publication" (Nakabo Edition, 2000). The Nakabo Edition (2000) also includes a "family search," but the figures are in monochrome. It would therefore be more efficient to search for species after establishing an approximate target for family and genus with another type of guide.

If identification is difficult, it is important to first reliably establish family and genus level rather than attempting to identify species level. If you leave photographs or record categorical traits it can be subsequently determined by an expert.

Sex: Sex is determined by external reproductive organs (penis, scrotum, vaginal orifice) or internal reproductive organs (testicles, ovaries, uterus). Because there are many species for which it is difficult to determine sex, if a bone specimen is prepared for such types, you should do your best to make a record of the sex of the specimen.

External measurement: Before preparing a specimen, take external measurements whenever possible. In Japan, research concerning estimated length of fish excavated from archaeological sites begun by Akazawa (1969), for instance, was the first research where recorded length of extant specimens existed.

Locality: Record the place where the specimen came from if you know for sure where it came from. Data concerning locality enables study of geographical variation of animals.

Specimen label: Oil that seep out from bone specimens can damage the label or make the data recorded on it illegible. Take measures to enable the label to be left for posterity along with the precious specimen. Our laboratory uses water-resistant paper or laminated labels.

(4) Preparation of specimens

Prepare specimens after identification and taking external measurements. Animal specimens include various types such as specimens immersed in liquid, dry specimens, stuffed specimens, bone specimens and fur specimens. The specimens most frequently used for zooarchaeology in the case of vertebrates are disconnected bone specimens; dry specimens are most frequently used for shellfish.

A. Shellfish

Boiling: Soft body such as flesh is removed from the shell. The boiling method is the most frequently used method of removing soft body. Because boiling opens the shell of bivalves, the adductor muscle (so-called "scallop eye") must be severed. In the case of univalves, boiling time differs according to the size. Small univalves of less than a few centimeters are boiled for about 1 minute, whereas large univalves with a thick shell may be boiled for at least 10 minutes. If individuals with thin shells are over-boiled, the shell may crack. Be careful not to boil too much. The shell may also crack if it comes directly in contact with the bottom of a pot that has been heated by fire. To ensure safety of the shell, position mesh over the bottom of the pot to prevent direct contact.

Flesh removal: It is not difficult to remove the soft body of bivalves if you cut the adductor muscle. In the case of univalves, care must be taken when removing flesh from spiral shells. Over-boiling will harden the soft body and make it difficult to remove from the shell. Be careful not to boil too much. If the flesh cools, it will be difficult to remove. Once boiled, grip with a towel and remove the soft body with tweezers as quickly as possible. The soft body of univalves will adhere to the 2nd and 3rd layers of columella from the aperture of the shell. Therefore, insert the tweezers slightly and remove the columellar muscle adhering to the shell. If removing the soft body with tweezers, remove slowly by twisting the shell rather than the tweezers. If the soft body breaks before it is completely removed, the remaining flesh can be removed by hooking with a wire or washing with water. If you are unable to remove the flesh, you can wash it after it decays or pour alcohol on it and stuff with cotton after draining and drying.

Not only seashells are unearthed from sites; the lids of univalves are also often unearthed. When removing flesh, therefore, remove the soft body from the lid as well to use as a specimen.

Wet/Dry: After removing flesh, wash with a toothbrush, etc., and dry thoroughly. In the case of univalves, be careful of water remaining inside the shell.

Other methods: Another way to remove flesh is to freeze and then remove after thawing. Yet another method is to remove moisture with alcohol with the soft body still attached and dry in that state. These methods are effective for small shellfish.

B. Fish

Boiling: If possible, collect the scales of fish specimens. Scales are also sometimes unearthed at sites. Caudal fulcrums of carangid fishes are often excaved intact. Soft tissue such as muscle is also removed by boiling. This includes the method of pouring hot water from a kettle on the fish and boiling the entire body in a pot.

If pouring hot water from a kettle, pour a little at a time. Remove the flesh of the boiled part and remove the bones. If you are not yet used to preparing specimens, pouring hot water along with a good understanding of the anatomical positioning is your best bet. The fish skull in particular consists of many bones, which can be roughly divided into the neurocranium, infraorbital bones, jaw skeleton, opercula bones, suspensorium, hyoid arch, shoulder girdle and pelvic girdle. These skeletal regions are connected by joints and ligaments and are often removed together with the bones they connect. Remove bones one at a time from the boiled portion with tweezers. You may do one side at a time so you can tell the skeletal regions are from the right or left side. While confirming the region, arrange the removed bones so you can tell the anatomical positioning.

If boiling in a pot, boil so that the specimen does not fall apart, because if it does, you will not be able to tell the anatomical positioning. Over-boiling may deform thin bones. When boiled to the proper degree, remove the bones with tweezers as was previously described and arrange so you can tell the anatomical positioning.

Other methods: Other methods include using insects and using chemicals to dissolve the soft tissue.
Chemicals used to remove soft tissue include proteinase A, papain, bioplase and medical instrument cleanser. Tasinase is currently unavailable. Familiar chemicals such as tailpipe cleaner, weak alkaline stratum or denture cleanser are also used. Such chemicals can remove flesh efficiently. Chemicals may however damage the bones themselves or cause them to deteriorate. Care must therefore be exercised when using chemicals.

Another method is to have larvae such as dermestid beetles (*Dermestidae sp.*) eat the soft tissue. Dermestid beetles, however, are also infamous museum insect pests that eat holes in animal and plant cultural properties, particularly the black carpet beetle (*Attagenus japonicus*) and varied carpet beetle (*Anthrenus verbasci*) are considered class A harmful insects (frequently harm cultural properties and are capable of causing serous damage). The place where specimens are to be prepared requires careful consideration when using insects to prepare specimens.

Drying: Dry specimens thoroughly to prevent fungus. Fats may seep out from completed specimens, depending on the species of fish. In such cases, boil again and dry thoroughly. If fats cannot be removed by boiling, they can be melted by alcohol, etc. The bones of small fish species may be translucent and therefore difficult to observe with the naked eye. Observation can be facilitated by light dyeing with an alizarin red S saturated solution.

Hard tissue requiring special attention: The skulls of fish contain hard tissue such as otoliths. Use a colander with fine mesh when washing the inside of the skulls; be careful not to lose. Not only species, but age and growth can also be determined from otoliths, and they are used for estimating fishing season.

C. Mammals

Preparation of specimens differs according to the size of the animal. Small mammals such as rodents have many parts that reduplicate those of fish. Thus, here we shall concentrate on preparing medium size and large mammal specimens.

Decortication / organ extraction: Remove skin by cutting between the skin and muscle with a scalpel while pulling the skin. In the case of large mammals, skinning is facilitated by the force of gravity if the carcass is hung up for decortication. Next place the carcass on its back facing upward and extract the organs. When doing so, determine the sex by checking the internal reproductive organs (testicles, ovaries, uterus).

Disconnection of joints: Separate the cranium and atlas, scapula and ribs, and coxal bone and femur with a scalpel. Roughly divide into cephalic region, trunk and front legs (left and right) and rear legs (left and right). Because the scapula and ribs are not connected, the front legs can be easily separated from the trunk by inserting a scalpel from the rear. The ligaments must be severed in order to separate the cephalic region and rear legs from the trunk. Insert the scalpel ventrally between the cranium and atlas and separate by severing the large ligaments (alar ligaments and apical ligament of dens). To separate the coxal bone and femur, sever the large ligament (femoral head ligament) that connects the acetabular notch and femur. When separating joints, be careful not to damage the bones. After severing the surrounding muscle as much as possible, the joint can be easily separated by pulling while twisting.

Excision of soft tissue: As a rule, muscle tissue should be excised for each separated region. Work is facilitated by removing as much muscle tissue as possible. It is convenient to make a photographic record or drawings of the relative positioning of the carpals and tarsals when dissecting.

Decomposition of soft tissue: soft tissue such as remaining muscle tissue is generally decomposed flesh removal. Methods of decomposing soft tissue include (1) boiling, (2) using chemicals, (3) using insects, (4) decomposition by immersion in water, and (5) decomposition by burying in the soil.

Since we explained methods (1) - (3) in the description of fish, here we shall talk about methods (4) and (5). These methods are effective for medium-sized and large animals that will not fit in a pot for boiling.

Method (4), decomposition by immersion in water, calls for decomposing remaining soft tissue by immersing the part removed in water. To do so, place water in a garbage can with a lid or large plastic container, close the lid tightly and let stand for a certain amount of time. When immersing in water, divide into regions or left/right and place in a cleaning net or drying net together with a label. The amount of time to leave the tissue immersed in water depends on climatic conditions such as temperature; in summer, it takes about a month for soft tissue to decompose leaving bones only. Water however evaporates rapidly in summer, so do not forget to replenish water when needed. Water should be replaced from time to time to prevent decomposition from being stalled by aerobic microorganisms. You should also note that tissue may not decompose in the winter, but may rather turn into an adipocere formation.

When the soft tissue decomposes, throw away the water and wash the specimen. Use a colander with fine mesh when draining or washing, and be careful not to overlook fine bones or isolated teeth that may get mixed with the putrid matter. Decomposing soft tissue involves issues such as foul odor and flies. A location where others will not be bothered must be found for the container. Problems concerning draining of the water used for decomposition must also be taken into consideration.

Method (5), decomposition by burying in the soil, similarly calls for dividing each region or left/right and placing in a cleaning net with a label and burying in the soil. If buried shallow, the specimen could be dug up by dogs or raccoon dogs. The specimen should therefore be buried somewhat deep. In order to mark the spot so you can find it again after the passage of time, it is important to leave a record of the position and depth it was

buried. Burying in a sandy beach will accelerate decomposition and facilitate treatment after digging the specimen up. The sand beach itself moves so there is danger of being lost. There is also a method that calls for placing sand in a large plastic container or garbage can with a lid and decomposing.
After decomposing soft tissue from specimens, wash off any remaining soft tissue with a toothbrush and rinse well with water. Just as with the method of decomposing by immersing in water, wash in a colander with fine mesh so as not to overlook small bones or isolated teeth.

Bone specimens of birds are basically treated the same as those of mammals. Boiling is the most common method of removing flesh from bones. Bird bones are however thinner and more fragile than those of mammals and require more care when handling. Bones particularly adhere to wings and tail feathers, and may be lightly scraped when decorticating.

Delipidation/bleaching: Depending on the specimen, fat may fail to be removed and rise to the surface of the bone. Medium-sized and large mammals captured in the winter in particular tend to contain fat, which tends to remain in the bones. Fat also facilitates growth of fungus. Boiling is the easiest way to remove fat from bones. Livestock species tend to contain lots of fat, and if the fat cannot be removed by boiling, immerse in an organic solvent such as alcohol or acetone. A 50:50 mixture of ether and alcohol or 2:1 mixture of benzene and methanol may also be used. Organic solvents are however inflammable and require precaution when handling and storing. To bleach specimens, immerse in a diluted hydrogen peroxide solution. Bleaching can harm bones. You should therefore exercise caution when bleaching. Rinse well after immersing in hydrogen peroxide solution.

Drying: It is important to dry specimens thoroughly before storing. If specimens are not sufficiently dried, fungus will grow after being put away.

Bones requiring precaution: The tongue contains bones called "hyoid bones." Be careful not to damage the hyoid bones when removing flesh from the skull. Male carnivores, chiroptera, and rodents are equipped with a baculum. Be careful not to damage the baculum. The baculum, for instance, is important for determining sex of buried dogs.

(5) Precautions concerning specimen preparation

Chemicals: Under ordinary circumstances, specimens can be prepared without using chemicals. If however chemicals are used, you must understand their characteristics and handle/administer them properly. Treatment of effluent must also be sufficiently considered.

In Japan, for example, alcohol and ether used for degreasing are classified as the dangerous substance kind 4 (flammable liquid), and hydrogen peroxide solution used for bleaching is classified as a dangerous substance kind 6 (oxidizing liquid).

Zoonosis: Handling animal carcasses, etc., required proper sanitation. You should use rubber gloves when preparing specimens in order to reduce the danger of infection. Apparatus used for preparing specimens must also be washed and disinfected. In Japan, the Ministry of Health and Welfare and Ministry of the Environment have prepared guidelines for infectious diseases common to both animals and human beings. The guidelines can be accessed on the websites of those ministries. You should browse through the guidelines before attempting to prepare specimens.

3. Storage/disclosure of extant specimens

In Japan, extant specimens are merely considered "tools for research" in zooarchaeology, and it has been pointed out that there is not much awareness of administration and disclosure of specimens. Extant specimens serve as evidence to support the basis of identification. Those used for comparison must be open to public inspection to ensure reproducibility of identification results. It is necessary to prepare extant specimens that offer both superior quality and quantity in order to enhance precision of identification. Extant specimens are also systematically arranged and stored so they can be utilized.

Currently in Japan, it has become difficult to pass on many materials of universities and graduate schools due to abolishment of the research system accompanying transfer of instructors. From the future perspective, it has become necessary to stably collect, store, disclose and use such extant animal bone specimens. The Environmental Archaeology Section of the Nara National Research Institute for Cultural Properties is publishing its specimen catalog in sequence so the animal bone specimens it possesses (NAC specimens group) can be widely used.

First let's use eaten fish as specimens!

In order to study animal remains, it is necessary to prepare bone specimens of extant animals. The significance of this is the same as that of producing stone tools in order to get a good understanding of stone tools.

There is no way to learn how to prepare bone specimens other than experience gained by trial and error. Even if you fail at preparing a bone specimen, you will gain precious experience for thinking about use of past animal resources. Even if records related to specimens are incomplete and are of low biological value, as long as the species of the specimen is clear, it is a great specimen for identifying species of animal remains.

First, let's prepare a bone specimen for identifying the species of a fish that has been eaten. At first, we should prepare a specimen from the species of fish provided in detailed drawings of the various skeletal regions. When preparing specimens, even if you don't know the region of the bone or whether it is from the right or left side, as long as it is from a major skeletal region, it can be determined from diagrams.

Preparation of fish specimens does not require special equipment or chemicals. Under ordinary circumstances all you have to do is boil in a pot. Zooarchaeology also requires a wide variety of bone specimens. In this case, you should refer to the methods of preparation provided herein.

Natural history museums in each place may also have bone specimens, so when preparing specimens, you may consult with natural history museums in your area.