Guidelines for the Procedure of Obtaining Mammal Specimens as Approved by the Mammal Society of Japan (Revised in 2009)

The Committee of Reviewing Taxon Names and Specimen Collections, Mammal Society of Japan

([ ]: notes by The Committee at 2015)

Today's challenges faced by wildlife research communities are complex, involving many issues on nature conservation and animal welfare. They also face various issues in society relating to their accountability for the credibility of scientific studies and their results, while adhering to professional criteria through original publication. A number of academic societies and related organizations whose members conduct scientific study on animals have established policies and guidelines pertaining to the handling of live animals and preserved animal specimens (e.g., Japanese Association for Laboratory Animal Science 1991; Japanese Society of Zoo and Wildlife Medicine 2003; Nature Conservation Committee and Editorial Committee of the Ichthyological Society of Japan 2004; Japan Ethological Society 2005; Science Council of Japan 2006). In the Mammal Society of Japan (MSJ), The Committee of Reviewing Taxon Names and Specimen Collections ("the Committee") instituted the first edition of the Guidelines for the Procedure of Obtaining Mammal Specimens ("Guidelines") under the leadership of Dr. Kazuhiro Koyasu in 2001 (Mammalogical Society of Japan Committee on Taxonomical Names and Collections, 2001; in Japanese). In the first paragraph, the original edition explains in detail the background information that led to the establishment of the Guidelines. Thereafter, our society has seen increasing nationwide interest in environmental issues, animal welfare, and zoonotic diseases. On top of this general trend, major revisions to existing wildlife regulations as well as enactment of new domestic legislation that directly affect wild mammal studies in Japan, such as the Wildlife Protection and Hunting Law and the Invasive Alien Species Act, have made the MSJ recognize the need for a timely revision to the Guidelines. Under these circumstances, in 2006 the Guidelines Revision Task Force was formed under the

Committee. Members of the Task Force are as follows, in alphabetical order of their last names: Masahiro Iwasa, Hideki Endo, Mariko Kageyama, Shin-ichiro Kawada, Kazuhiro Koyasu, Motoki Sasaki, Keisuke Nakata, Masaharu Motokawa, and Yasushi Yokohata (Task Force Chair). The revised version was approved by the MSJ Council in July 2009.

The purpose of the Guidelines is to provide professional standards by which handling procedures of specimens in mammalogical research, including field collection methods, can be rightly evaluated as endorsed by the MSJ. Given the fact that the capturing of wild mammals in the field normally precedes preparation of specimens, the Guidelines address not only field research techniques but also ethical considerations from the standpoints of animal welfare and conservation. As far as the handling of live animals is concerned, the MSJ has not yet adopted procedural guidelines as of 2009. Therefore, similar guidelines developed by some other organizations (e.g., Japanese Association for Laboratory Animal Science 1991; Fukui 1991; Japanese Society of Zoo and Wildlife Medicine 2003; Japan Ethological Society 2005; Science Council of Japan 2006; outside Japan, Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987; Bookhout 1996; Animal Care and Use Committee 1998; Gannon et al. 2007) should be referred to where appropriate. Ethical aspects of conducting research using live animals are outlined in Murakami and Saeki (2003).

The Guidelines are not intended to discourage researchers from attempting to undertake a project using a revised and improved methodology or from designing an original research project using a new technique. Ultimately, each researcher must be responsible for their decision as to which project design and method they adopt. Intended users of the Guidelines are basically MSJ members and other non-member mammalogists. However, it is highly expected that the Guidelines are of interest to other stakeholders, such as natural history museums with no mammal expert staff, policymakers and government agencies that deal with legal regulations on mammals, foundations and support organizations that provide research grants to mammalogists, as well as to editors and reviewers of academic journals that accept papers in the field of mammal research.

1. Value of Specimens in Mammalogy

Today, mammalogy encompasses a broad range of research hypotheses as well as various approaches to test them. When the subject of research includes wild mammals, it is often the case that you have to live-capture or kill animals in order to obtain necessary data from the study material. The information obtained enables accurate identification of species and an understanding of systematic and evolutionary relationships, genetic phenomena, population dynamics, community structure and dynamics, comparative anatomy and physiology, behavior, parasites and diseases, economic importance, geographic and microhabitat distributions, ecology of mammals in their natural or managed environments, and other scientifically important phenomena (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987). In reference to other mammalogical societies outside Japan, for instance, the American Society of Mammalogists strongly recommends that researchers donate various types of mammal specimens obtained during field studies to "systematic collections that meet minimal standards" (Committee on Systematic Collections 1975, 1978; Systematic Collections Committee 2004; see Appendix to the Guidelines for a Japanese translation of the latter, which is omitted in this English version) as vouchers, after the completion of the study. A collection in a museum that meets such standards serves as a permanent repository of voucher specimens and their associated data examined by different researchers. Voucher specimens preserved in this manner subsequently constitute a systematic collection that enables validation of past research, and that also serves as a resource available for present and future research. Abe (1992a) goes into detail about the significance of voucher specimens collected and preserved in mammalian research from the angles of taxonomic information, ecological and physiological information, and other ancillary information. Maximizing the scientific value of specimens entails adequate preparation, documentation, and preservation of original specimens and their associated data. The Guidelines aim at providing professional benchmarks to standardize procedures of collecting and preparing mammal specimens, and for documenting associated data. The Guidelines are also meant for mammalogists to serve as a practical guide in preparing specimens. Furthermore, topics of professional ethics and techniques related to wild mammal collections discussed herein would be relevant to ecologists in general.

2. General Issues Concerning Mammal Collection in the Field

Sampling protocols for mammal specimens include not only traditional lethal means of collecting museum specimens, but also other nonlethal approaches such as obtaining biopsy samples from live-captured individuals and releasing them afterwards. The Guidelines mainly take into account ramifications of adopting lethal methods. Hamazaki (1998), Kishimoto (2002), and Kaneko and Kishimoto (2004) point out "safety of animals," "safety of workers," and "minimal impact on the environment" as "three basic rules of animal collecting." In this context, emphasis is made on the need for compliance, significance of the rules, and some other points of consideration. Despite originally being proposed primarily with nonlethal methods in mind, these three principles are also applicable to lethal methods in general. Consulting the Handbook of Japanese Wildlife Care and Medicine Editorial Committee (1996) and Bookhout (1996) should be required to reference the technical aspects of stabilization, rescue, and external measurement of data collected from live-captured wild mammals.

2–1. Laws on Field Collecting

The following discussion is based on Ikeda and Hanai (1988), one of the first review articles that outlined regulations and legal procedures that are relevant to field mammal collectors in Japan. The topic is divided into three main sections, namely, Wildlife Protection and Hunting Law, more commonly referred to as the "Wildlife Protection Law (WPL)" (original Japanese title, *Choju Hogo oyobi Shuryo ni Kansuru Houritsu*, subsequently changed to *Choju no Hogo oyobi Shuryo no Tekiseika ni Kansuru Houritsu*) [currently, Wildlife Protect Management Law (WPML), *Choju no Hogo oyobi Kanri narabini Shuryo no Tekiseika ni Kansuru Houritsu*]; Law for the Protection of Cultural Properties (*Bunkazai Hogo-ho*), and other relevant laws. WPL experienced a major revision in 2003, in which the term "wildlife" (= *Choju*) was distinctly defined as "wild animals that belong to Aves and Mammalia." This definition allows for further inclusive application of the existing law to all the Japanese mammals encompassing the groups that were previously placed outside the scope of the law: order Insectivora [currently, Soricomorpha and Erinaceomorpha], family Muridae (excluding pests caught by agricultural and forestry workers in addition to three residential species: *Mus musculus*, *Rattus rattus*, and *R. norvegicus*), and certain taxa of sea mammals (i.e., seals, sea lions [not including

Steller Sea Lions], and dugongs; see below for the other marine mammals that remain exempt from said regulation) (Ishinazaka 2003; Yokohata 2003). In order to collect any wild mammal species regulated by the WPL for research purposes, either one of the following two types of collection permits is required: Wildlife Collection Permit issued under WPL Clause 1, Article 9, or Class-A Hunting License issued under WPL Article 39 [currently, also see WPML Articles 9 and 39]. Note that the latter is more restrictive in the conditions than the former in that a permit holder is allowed to catch only those species listed as game animals using regulated tools and equipment only, within a restricted hunting zone, for a limited period of the hunting season. The following reference is useful, besides the aforementioned, in acquiring information before applying for a new collection permit for scientific research purposes: Komaru (2001), Wildlife Conservation Administration Study Group (1992, 2003), and Wild Bird and Mammal Management Study Group (2001). For further details, first consult the appropriate governmental agencies responsible for issuing collection permits. Hatakeyama (2004) and Sakaguchi (2007) recently added to the literature on this subject.

Alongside the general laws discussed above, collection of Japanese mammals that are listed as threatened species are now regulated under the Endangered Species of Wild Fauna and Flora Conservation Law that was issued in 1992 and has been enforced since April 1, 1993 (Environmental Agency Wildlife Conservation Administration Study Group 1993, 1995; Management and Coordination Agency Administrative Inspection Bureau 1993). International regulations pertaining to the export and import of specimens of endangered species are implemented by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) as discussed in the section 6–2.

To summarize, collecting, importing, and/or exporting wild mammals are subject to domestic and international regulations based on the wildlife laws and treaties, such as those stated earlier; therefore, if certain species, locations, or activities related to your fieldwork are subject to these laws, obtaining valid permits issued by authorized agencies is mandatory. These laws and acts may be amended with addition of new regulations in the future; hence, each researcher is responsible for being versed in all the regulations that affect their study area and for acquiring necessary licenses ahead of time (Rohlf 1995; Wildlife Management Study Group

2001; Wildlife Conservation Administration Study Group 2003; Hatakeyama 2004; Sakaguchi 2007).

Among Japanese marine mammals, the collection of whales and Steller Sea Lions is regulated under the Fisheries Act, and certain species of whales are also stipulated in the Fisheries Resource Protection Law. In order to legally collect these animals for scientific study, a researcher must get permission from the Minister of Agriculture, Forestry, and Fisheries based on the Enforcement Regulations of the Fisheries Act, Article 1, or the Enforcement Regulations of the Fisheries Resource Protection Law, Article 1. Likewise, collection of domestic sea otters and fur seals is basically prohibited by the Sea Otters and Fur Seals Hunting Control Act. For scientific collection of these groups of Japanese sea mammals, persons must obtain a license from the Minister of Agriculture, Forestry, and Fisheries following Article 1 of the Enforcement Regulations of the above law.

In addition to field collection permits, persons may also need to consider seeking approval related to public and private land use. For instance, persons need to apply for a forest use permit granted by a district forest office as a requirement for entering a national forest, and in the case of a public land, they are obligated to obtain permission from a land owner and manager ahead of time, before they collect wildlife on their land. Furthermore, the Invasive Alien Species Act (issued in 2004, enforced since October 1, 2005) prohibits the raising, possession, and transferring of Invasive Alien Species, including 16 species [currently 19 species and 2 hybrids] and 4 genera of mammals (as of 2008) in principle. Komaru (2001) provides general guidance on biological fieldwork and relevant laws, which include a listing of contact information for inquiries about the field sampling of organisms.

2–2. Ethical Issues on Field Collecting

Ethical issues regarding wildlife sampling are clearly illustrated by Nagorsen and Peterson (1980). The possession of a valid collecting permit does not give the collector the right to collect animals irresponsibly. Persons are supposed to conduct fieldwork using the most humane method possible and strive to minimize impact on the local biota without disturbing sampling sites. Do not engage in indiscriminate mass collecting. This is not permissible, especially in circumstances where a large number of animals are clustered in one area. If possible, it is advisable to live-capture mammals, and as soon as the total number reaches the needed sample size, release

excess live animals to the wild intact. Such flexibility is only possible when live traps are used, and there is no such option if persons conduct fieldwork using kill traps. In fact, the sample size required for a certain study is subject to the study design. Therefore, every investigator who conducts fieldwork is held accountable for a particular sample size adequate for their study at their own discretion, and they should collect no more specimens than are needed (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987; Animal Care and Use Committee 1998; Gannon et al. 2007). On the other hand, it is also inadequate if too few animals are sampled because this might not allow the investigator to achieve the study purpose and, subsequently, will result in waste of animals (Gannon et al. 2007).

Friend et al. (1994) justifies with clarity the need for adopting lethal methods in wildlife study as described in the following statement: "[The] collection of animals often is an essential component of field investigations. These studies may involve systematic zoology, comparative anatomy, disease assessments, food preference studies, environmental contaminant evaluations, and numerous other justifiable causes and scientific needs." To evaluate a proposed study requiring animal collection, Friend et al. (1994) further enumerates the following criteria of assessment: 1) whether the proposed collection will provide scientific data that is not duplicative of information already in the scientific literature, or information that is presently available in accessible scientific collections and repositories; 2) whether suitable information can be obtained from alternative methods that do not require taking live animals; 3) whether methods of collection are acceptable and effective to minimize the potential of trapping non-target species; 4) whether target animals can be killed as quickly and cleanly as possible; and 5) whether appropriate methods are employed to be as age-class specific as possible according to the purpose of the study. Finally, specimens intended to be preserved in a museum to build a systematic collection must be prepared with meticulous care, and scientific data associated with specimens must be documented and archived using a standard method (see 4–1 through 4–7).

3. Animal Collecting Equipment and Techniques

3–1. Collection Using Kill Traps and Guns

When collecting mammals using kill traps, special attention should be paid to not let animals suffer unnecessarily nor damage their body parts that are to be saved for the study. An all-plastic snap trap model, commercially known as a "Panchu trap" in Japan, is one of the most convenient and easiest to use for catching small mammals, such as small rodents and terrestrial insectivores (Abe 1991a, 1992b; Murakami 1992; Yoneda et al. 1996). Other brands of snap traps include a Victor® trap and various other types of metal traps, but these models, excluding a Museum Special (made in USA) and a custom-built metal snap trap (see Murakami 1992), are prone to crushing skulls (Imaizumi 1970), and therefore, may require a slight adjustment to the device such as repositioning a bait trigger plate. Murakami (1992) provides source information about these products. As for catching fossorial moles, various types of mole traps are introduced, including a scissor trap, a tube trap that comes with a wire loop for strangling, and a harpoon trap (Abe 1992b, Yokohata 1998). Specifically for water shrews, a large-size snap trap with a bait trigger plate, a tube trap attached with a mesh screen, as well as a "mujiri" cage traditionally woven from reed or bamboo, have been used (Abe 1992b). Using these kill traps is considered an adequate means of collecting small animals that meet a research purpose as well as an ethical standard.

Decisions regarding a trapping method and trap positioning should be made based on a factor that maximizes the odds of catching the intended species and minimizes the chance of trapping non-target animals. To avoid overlooking traps, it is important to mark the site of each trap station with a marker like colored tape. In addition, a collector is required to put a label on each trap that includes information such as the permit holder's name, a name of the office issuing the permit, and a permit ID number. In case a trap is too small to label, a collector must post a placard or a sign at the site. It is essential to check all the traps at least once a day, ideally early in the morning, to remove trapped animals as soon as possible. Mammal carcasses tend to decompose rapidly at high temperatures, and if they are left in traps long enough before being picked up, they could be prone to ant damage or maggot infestation. Pitfall traps are particularly suitable for collecting shrews (Abe 1992b; Koyasu 1998), but if this type of trap is used as a kill trap,

it should contain sufficient water to ensure that the captured animal(s) do not suffer long before drowning (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987).

Smaller-size "body-gripping traps" have been in use as kill traps for chipmunks and tree squirrels (Abe 1991a) as an exceptional case, but generally body-gripping traps and comparable foothold traps are known as typical non-humane models that do not kill animals quickly. Therefore, alternative and more humane types of traps should be chosen when available. If there are no substitutes for steel jaw traps, it is recommended that modified traps with padded jaws be used (Kuehn et al. 1986; Nakazono and Doi 1989; Ikeda 1989; Kaneko and Kishmoto 2004), and that traps be checked frequently, at least twice a day (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987).

For mammals such as rabbits, hares, squirrels, and small- to medium-sized carnivores, shooting may be more humane than trapping (Nagorsen and Peterson 1980). In order to use guns for scientific collecting by the researcher, they are responsible for obtaining a hunting license beforehand, specifically, the first-class gun hunting license for use of firearms and air guns [currently, firearms only], or the second-class gun hunting license for air guns only, as set forth in Article 39 of the WPL. On top of that, they must abide by regulations on the possession and use of guns known as the Act for Controlling the Possession of Firearms or Swords and Other Such Weapons, or more commonly referred to as the Swords and Firearms Control Law (Japanese title: *Juhou Touken-rui Shoji-tou Torishimari-hou* or "*Jutou-hou*"). In addition, there is a law that specifically regulates the possession and storage of ammunition called the Explosives Control Act (*Kayaku-rui Torishimari-hou*). Researchers must comply with these laws and gain sufficient experience to use guns properly and safely.

For the scientific collecting of large cetaceans, a whaling gun loaded with an explosive harpoon is used. A harpoon gun charged with an explosive called penthrite (pentaerythritol tetranitrate or PETN) is recognized by the International Whaling Commission as one of the most humane killing techniques for whales (International Whaling Commission 1981). In the case that an explosive harpoon did not kill a whale instantly, a 9 mm (0.35 in) caliber or a larger caliber rifle may need to be used as a secondary means of finishing. Cetacean species are not subject to the WPL, and therefore, regardless of the method of killing whales, a license is required for the possession of

firearms in accordance with Article 4, Paragraph 1, Item 2 of the Swords and Firearms Control Law.

3–2. Collecting Using Live Traps and Nets

Live trapping is often a preferred method of sampling mammals in certain areas of research such as karyotyping, biochemical and genetic studies, ectoparasites, and a mark-release-recapture approach in ecological studies. One of the merits of using live traps is to enable a researcher to capture only the target species and number of specimens needed for study, and to release all the other unwanted animals to the wild unharmed. However, it is important to choose the right type of live trap that works most efficiently for its intended catch. For example, it is known that the trappability of *Crocidura* shrews is significantly higher than that of *Sorex* shrews when Sherman-type live traps are used. Live traps are usually made of box- or tube-shaped containers using various materials such as aluminum, zinc, wood plates, wire mesh, and plastic. They are designed to work in a manner in which an animal that is lured into a trap container would come in contact with a trigger inside to close its door in a set position, resulting in the live animal contained in the trap. Commercially available brands include Sherman, Havahart, Longworth, Penlon, and Tomahawk, and live traps can be ordered either directly from these overseas manufactures, through domestic retailers (see Murakami 1992), or by international mail order (e.g., Carolina Biological Supply Company; www.carolina.com). Products similar in shape and function to those foreign brands are manufactured and sold in Japan as well (Murakami 1992; Yoneda et al. 1996). Penlon-model live traps made of plastic are capable of trapping small to medium-sized mammals such as shrews and large field mice (Shibanai and Izeki 1997), whereas wire-mesh traps are usually used to capture live, larger-sized mammals such as nutrias (Miura 1992) and wild cats (Izawa 1990). Simple techniques of collecting live moles include live-capture by ambush or with pitfall traps, but tube-shaped live traps, such as the Nishi-method and the Konishi-method, are more reliable and are often used by mole researchers (Abe 1992b; Yokohata 1998; Kawaguchi 2004). An overview of live-trapping techniques for soricid species is found in Abe (1992b), Koyasu (1998), and Motokawa (1998), whereas trapping techniques for carnivores are summarized in Kaneko and Kishimoto (2004). Wooden box traps are

preferably used for mustelids in order to keep trapped animals from damaging their teeth in their attempt to break a metal trap open (Sasaki 1990). In the case of live-catching large-sized mammals using foothold traps, the jaws must be cushioned or padded with rubber for softer contact as discussed in the preceding section (Ikeda 1989). Giant flying squirrels can be caught using a modified sweep net (Baba 1988). Box traps or snare traps are often used to catch live hares (Yamada et al. 1988).

It is important to choose a live trap of an appropriate size large enough to allow for movement of the target species without its suffering inside the trap. During the cold season, in order for the animal to avoid exhaustion due to decreased body temperature, it is imperative to place a sufficient supply of food and nesting material inside the trap, and to cover the whole trap with heat insulation material. Each live trap should be regularly checked at an adequate frequency to minimize accidental death or exhaustion of the animal trapped inside. When live-capturing large-sized mammals using box-shaped traps, persons may not need to visit traps so often if a radio transmitter is attached to the trap's door. It usually works best if a transmitter is kept turned on while the trap door is set open, and the signal is automatically turned off as soon as the door is shut. The opposite mechanism of keeping a radio device off when a trap is set open (and turning it on upon the capture of an animal) is not recommended because of higher risk of mechanical failure. Live traps for nocturnal species should be set before dusk and checked as soon as possible after dawn. They should be closed during the day after the morning check to prevent accidental capture of diurnal species (Animal Care and Use Committee 1998). Live traps for diurnal species should be shaded, so as to avoid full exposure to the sun and should be checked frequently (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987; Koganezawa 1989).

During routine walks, attention should focus on checking traps, so as to not overlook any live animals caught in traps. Further, at the end of fieldwork, make sure all traps are retrieved and temporary field markers, such as colored tape, are completely removed from the study area. It is recommended to label all traps with sequential numbers and keep track of them in numerical order during the initial setup, regular checking, and final retrieval. In this way, one can reduce the chances of overlooking any

captured animals during a regular trap inspection or leaving behind a trap at the end of a trapping session.

Highly frequent inspections are required when live-capturing small mammals using pitfall traps (e.g., about once every hour for shrews). Bait should be placed in a pitfall trap in order to keep trapped animals from starving to death. In addition, captured animals experience a higher mortality rate when they are wet from rain, so it is necessary to cover the top of a pitfall trap to provide shelter for the trapped animals (Pucek 1981).

To live-capture large-sized mammals, various types of live traps are conventionally used. For bears, box traps (box-shaped cages: Watanabe and Nozaki 1989; Kaneko and Kishimoto 2004) and barrel traps (drum traps: Mano et al. 1990; Yoneda et al. 1996; Kaneko and Kishimoto 2004) are commonly used. For artiodactyls such as sika deer, Japanese serow, and wild boar, box traps and net traps are known to be effective, as well as a corral trap system, in conjunction with a metal fence structure, to drive animals into an enclosure (Ito et al. 1989; Nakatani 1989; Kaji et al. 1991). Specifically for deer, a modified corral trap system is adopted, by which the deer would be enclosed by cloth curtains that rise when the animals enter the enclosure (Uno et al. 1996). As for wild boar, snare traps are also conventionally used (Nakatani 1989). When the two models of bear traps are compared, one of the disadvantages of using metal cages is that a captured bear likely ends up getting its teeth damaged from biting the metal bars of the cage (Yoneda et al. 1996). In contrast, a bear is unlikely to be injured when captured in a barrel trap (Mano et al. 1990). Likewise, when a metal fence corral trap is used for catching deer, there is a higher chance that the animal would crash into the metal fence, leading to major injury or death. On the other hand, an improved corral trap system outlined with soft cloth screens is considered much safer for capturing deer (Uno et al. 1996). Today, snare traps are deemed inappropriate to capture live wild boars because of higher injury and mortality rates (Nakatani 1989).

When live-catching large wild animals, it is essential to perform various necessary procedures in as short a time period as possible after the capture or the animals may become violent and be injured. It is also important to pay careful attention to each animal that is captured and immobilized in order to avoid causing capture myopathy (CM). One has to be prepared to be ready to administer a fast-acting treatment against CM symptoms

(Suzuki 1999). From a legal standpoint, a hunting license or a scientific collecting permit is required for live-capturing wildlife, specifically a net hunting license and a trapping license are required when using nets and live traps, respectively. Additionally, in the case of using firearms and air guns, you must comply with laws and ordinances that regulate the possession and use of these devices (cf. Ikeda and Hanai 1988). If an anesthetic is applied in order to live-capture animals, it is mandatory to obtain permission from the Environment Minister to use an anesthetic designated as a deleterious medicine (cf. WPL Article 15 [currently, Article 37]).

As far as bats are concerned, a collection permit is required to capture bats for scientific research purposes. In addition, permission must be obtained from the Environment Minister in order to use a mist net, because the law generally prohibits the sale, possession, or use of mist nets (cf. WPL Article 9, Paragraph 1, Item 3). Further, it is required to present the above collection permit in order to buy a net (WPL Article 16, Paragraph 2) [currently, WPML Article 16, Paragraph 2, Item 1]. The Bat Study and Conservation Group of Japan (1998) provides useful information on how to purchase a mist net, as well as techniques for using it to catch bats. It is important to adopt the most appropriate capturing method for different species of bats depending on specific habitats they live in (Mohri 1988; Kunz and Kurta 1988). Researchers generally should avoid exploring a cave where a bat maternity colony is roosted during the period of parturition and nurturing, so as not to disturb the breeding colony. Repeated disturbance of hibernating bats may also lead to higher mortality (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987) and must be avoided. There is a special device known as a “One-Way Door” that can be installed to force a roost out of a residential structure (Gelfand 1997; Tuttle and Hensley 2000). However, One-Way Doors should not be installed during the parturition and nursing period as this may lead to higher mortality of flightless pups, especially if this method is applied in the summer breeding months of June, July, and August. A collection permit is not required to build a bat house (a shed or a box designed to shelter a bat roost) as far as it is only meant for conservation and observation of bats, and the project does not involve direct handling of bats. However, needless to say, one should get permission from a landowner and property manager prior to building a bat house on their property. In addition, if fieldwork entails temporary or

permanent capturing of bats from a bat house for data gathering, a collection permit is required to conduct such a scientific study. Choosing the right kind of bat house with respect to its materials and size, and selecting a suitable location to install a bat house is important for its effective use. It is advisable to provide additional devices in the area that protects bats from attacks by potential predators (Gelfand, 1997). Overall, bat research requires familiarity with the research methodology peculiar to this group of mammals, including trapping techniques, and therefore investigators should be well versed in ecological and behavioral study techniques (cf. Kunz 1988) for the specific group of bats of interest.

Encircling nets or hoop nets are usually used to live-capture smaller-sized cetaceans, especially dolphins and porpoises. The former method has the advantage of capturing a school of marine mammals effectively at one time, but necessitates operation of multiple vessels in coordination. The latter method takes advantage of the dolphin's habit of approaching a sailing ship to ride the bow wave (Ridgway 1972), but the technique is hardly adopted in Japan. In either way, full attention is needed during the lifting and transport of animals to an adequate aquarium facility to minimize undue stress.

When euthanizing a live mammal captured in the wild, the most ethical and humane way must be followed (as a legal justification behind this statement, nonbinding rules regarding animal euthanasia are stipulated in the Articles 23 and 24 [currently, Articles 40 and 41] of the revised Act on Welfare and Management of Animals; see Animal Welfare and Management Laws Study Group 2001). In general, euthanasia, or mercy killing, means the act of putting an animal to death without inflicting prolonged suffering by rendering it unconscious in the shortest time possible (Japanese Association for Laboratory Animal Science 1991). Administratively, animal euthanasia "shall follow chemical or physical procedures that are the most effective methods possible to trigger the loss of consciousness without causing pain or distress to a sacrificed animal, resulting in irreversible cessation of its cardiac or lung function, or follow other socially acceptable methods in a normal manner," according to the Guidelines on Methods of Sacrificing Animals (Notice No. 40 of the Japanese Prime Minister's Office, 1995 [currently, No. 105, 2007]; see also Asano et al. 2006). Various agents and methods of euthanasia are known such as administering an overdose of anesthetics, ether, or carbon dioxide (Tajima

et al. 1979; Nakamura et al. 1984; Japanese Association for Laboratory Animal Science 1991; Japanese Prime Minister's Secretariat Management Office 1996). Selecting a technique should be based on an evaluation of the most humane method that, if at all possible, would not subject the animal to pain or suffering while meeting the study purpose. Administration of ether used to be one of the most frequently adopted procedures, but today ether is not recommended because of its toxicity to humans and animals, as well as its irritative, explosive, and flammable attributes. On the other hand, the use of carbon dioxide as a method of euthanasia can cause irritation of mucosal linings in some species (Gannon et al. 2007). Euthanasia must be performed outside the perceptive range of other animals so as not to trigger fear or undue stress in other captive individuals (Gannon et al. 2007). If you have any questions or uncertainty regarding euthanasia techniques for particular study animals, it is recommended to consult veterinarians or laboratory animal experts who are knowledgeable about euthanasia protocols for domesticated or laboratory animals. The latest review on the ethical perspective of animal experimentation is found in Kagiyama (2008).

4. Data Associated with Specimens

It is recommended to store field data associated with mammal specimens that were collected, examined, and preserved for systematic study, as well as for other research purposes. For instance, field data in conjunction with specimens that were originally captured for field surveillance can further provide a wide variety of biological information, such as geographic variation in morphology, age variation, age determination, and age structure in a population, estimation of the breeding period based on age determination, reproductive condition, litter size, diet and feeding habits, nutrition status, quality of a population, and parasites (Abe 1991b). Moreover, in closely related study areas, such as morphology, genetics, biochemistry, and parasitology, preservation of study materials as voucher specimens not only ensures the taxonomic underpinning of a study, but also makes it possible to correspond with future taxonomic revisions. Therefore, whatever the main study purpose of collecting mammal specimens could be, documentation of basic field data for each specimen (e.g., collector's serial number, species identification, sex, measurements, locality, date collected, method of collection,

other remarks) should be accomplished as a part of the daily tasks of fieldwork, and such primary records should be archived together with physical specimens. However, uncertain data should never be recorded based solely on speculation even if an investigator is caught up with taking multiple specimen data. For example, if the sex of a specimen cannot be determined in the field, it is better to indicate so with a question mark (?) in a field notebook or a field catalogue (Nagorsen and Peterson 1980).

4–1. Recording Data

It is most common that data associated with field collecting activities is recorded in a notebook or a certain data form. Martin et al. (2001) recommends that field notes be organized into three separate sections: 1) a diary journal, 2) field catalogue, and 3) species accounts. In the journal, all collecting activities and daily observations are documented as a field diary on a day-to-day basis. The field catalogue section is useful to record specimen data, such as measurements, for each animal collected, and such data is recorded in the order of the collector's serial number assigned to each specimen. The species accounts section includes detailed observational records on particular species that are captured (Martin et al. 2001). It is important to archive a diary journal generated for all types of fieldwork. It is also advisable to document a field catalogue on printed catalogue sheets rather than a blank notebook (British Museum 1968; Nagorsen and Peterson 1980). Unless custom printed catalogue sheets are used for a field catalogue, commercial A4-sized horizontal-ruled data-recording sheets (e.g., KOKUYO Accounting Pad SHIYO-26N) are recommended for use as catalogue data forms (catalogue sheets) given their ease of use and reasonable price. Today it is acceptable to record field data electronically using spreadsheet software such as Microsoft Excel, but data on electronic media should be backed up, ideally printed out on paper to be filed physically, to ensure long-term preservation of the original data.

Data categories that appear as column headings on a catalogue sheet are as follows:

Field Number–this is a unique number assigned to each specimen that is recorded on a catalogue sheet in the field. Unless one already follows their established specimen numbering system, it is advisable to stick to the collector's serial numbers, that is, a series

of the collector's sequential numbers independent of field trips, localities, species, or collection dates. The field number should be permanently written on a field tag attached to each specimen with a pencil, India ink, or permanent ink, but never with a ballpoint pen, felt-tip marker, or a fountain pen loaded with a water-based ink cartridge.
Catalogue Number–a number that is assigned and recorded once the specimen has been accessioned to an institutional collection; thus, catalogue numbers are not recorded in the field.
Species Name–Record of species identification (Japanese name or scientific name) in the field. Useful references for identification of Japanese mammals include colored illustrations (Imaizumi 1960), keys (Maeda 1983; Yoshiyuki 1989; Abe et al. 1994, 2005), and skull illustrations (Abe 2000).

Standard formats for recording Sex, Weight and Measurements, and Locality are discussed below under the sections 4–3, 4–4, and 4–6, respectively. Information on Reproductive Condition, Habitat, and Field Observations are documented either as three separate data categories on a catalogue sheet or can be lumped together under "Remarks" (see sections 4–5 and 4–7).

When specimens are to be deposited in a museum collection, the documentation associated with them should also be permanently archived by museums, including all catalogues, field notes, photographs, and maps of collecting sites. As catalogue sheets and field notes are often the only source of information for specimens, the catalogue and field notes should be well organized, legible, and as accurate as possible (Nagorsen and Peterson 1980).

4–2. Specimen Labels

Choose the right kind of quality paper that is resistant to alcohol and formalin solutions for specimen labels. Labels are generally attached to all different types of preparations: study skins, skulls, skeletons, and specimens in fluid. Writing the collector's initials preceding the collector's field number on a specimen tag helps to prevent any possible confusion with specimens of another collector (Nagorsen and Peterson 1980). Refer to Abe (1991b) for data items to be recorded on a specimen label. In essence, this means transcribing each data item as completely as possible from the

catalogue onto a label. Some museums, like the Royal Ontario Museum in Canada, adopted a computerized cataloguing system, in which a specimen is originally labeled with only a field number tag (Nagorsen and Peterson 1980). This approach is deemed effective at institutions where a collection data management system is well established and flawless so that there is no chance of issuing duplicate labels. Determine data items to be included on a specimen label as well as its format and style following examples provided in references such as Okada (1940), British Museum (1968), Imaizumi (1970), Nagorsen and Peterson (1980), Martin et al. (2001), Pucek (1981), Handley (1988), and Yoneda et al. (1996). A set of skin, skull, and other associated specimens, such as tissue samples obtained from an individual, should be assigned the same specimen number even if they are stored at different locations. When molecular and cytological specimens or host specimens for parasitological inspections are provided to outside institutions and researchers, associated specimen numbers should always accompany the samples being given. In published literature, numbers for voucher specimens, and tissue or parasite samples should be cited for cross-reference.

4–3. Sex Determination

It is often possible to determine the sex of an animal based on an inspection of the external reproductive organs, such as the vaginal opening, scrotum, and penis, for medium to large-sized mammals, as well as for small mammals if they are in the breeding season (Imaizumi 1970; Abe 1991b). In the case of cetaceans, external reproductive organs are housed within a genital slit, but it is possible to tell male from female based on a difference in the distance between the anus and the genital slit, which is relatively longer in males than in females (Nagorsen and Peterson 1980). Similarly, rodents can be sexed externally on the basis of the distance between the anus and the reproductive organ, specifically, the clitoris or penis (Nakata 1986; Abe 1991b). On the other hand, juveniles and small mammals in the non-reproductive season have poorly developed external reproductive organs. Therefore, it is advisable to inspect the internal reproductive organs of a specimen to reduce the chance of false sex determination (Imaizumi 1970; Nakata 1986; Abe 1991b). In order to sex a specimen by its internal organs for reproduction, the ventral side of the body has to be dissected, normally after it is skinned to prepare a study

skin. Sex can be determined by verifying the presence of testes for males and the uterus for females. Insectivores, such as shrews in a non-reproductive condition, demonstrate fairly reduced internal reproductive organs. However, you can confidently differentiate male from female soricomorphs by verifying the presence of testes located at each distal end of a pair of vas deferens extending toward the right and left from the base of the urinary bladder for males and the presence of a T-shaped uterus positioned at the upper dorsal side of the urinary bladder for females (Abe 1991b). In order to sex fetuses or carcasses of wild mammals, analytical techniques at an intracellular level (e.g., chromosomes, DNA) are necessary (Dimmick and Pelton 1996). See Koyasu et al. (1995) for examples of the application of such techniques in Japan.

4–4. Body Weight and External Measurements

Specimens should be weighed as promptly as possible before beginning preparation. Weights are measured in grams for small mammals and in kilograms for larger mammals. A scale is used for weighing small-sized animals, and a portable digital scale (e.g., Tanita Mini Scale 1476 model) is convenient to use for animals that weigh up to 100 grams. It is important to carry backup batteries with you whenever using a battery-powered scale in the field. The body weight of a large mammal is difficult to obtain in the field. Nevertheless, the weight should be recorded whenever possible because of the paucity of such field data for large animals (Nagorsen and Peterson 1980). Normally, a portable mechanical spring scale is used for weighing large mammals in the field. When a power source is available, an electronic livestock scale is useful (e.g., Tru-Test Distributers Limited [currently, Tru-Test Limited], New Zealand, AG and EZ models [currently, these models may be outdated and no longer available on the market.]).

Standard external measurements for small mammals are, in the North American method (as established in Canada and the United States), Total Length (TL), Length of Tail vertebrae (LTv), Hind Foot length (HF), and Ear length (E) (Corbet and Ovenden 1980; Nagorsen and Peterson 1980). In this system, hind foot length includes the extent of the claw or nail without exceptions (Hfcu: Hind Foot cum unguis). In contrast, in Europe including the United Kingdom, Head and Body length (HB) is more commonly measured instead of total length, and hind foot length is not supposed to include the

extent of the claw or nail (Corbet and Ovenden 1980; Nagorsen and Peterson 1980; Pucek 1981; Imaizumi 1986). When measuring tail length, there are differences between countries in defining its point of origin. In the United Kingdom and North America, the distance from the root to the tip of the tail is measured (Imaizumi 1970, Corbet and Ovenden 1980; Handley 1988; Burton 1991), whereas in the rest of Europe outside United Kingdom (e.g., Poland), tail length is defined as the distance between the center of the anus and the tip of the tail (Length of Tail anus: Lta) (Pucek 1981). In either system, a tuft of hair extending beyond the end of the tail is excluded from the measurement. Formerly in Japan, both the European method and the "amended" North American method (tail length measured in the North American way versus hind foot length measured in the European way) have been in use, but in more recent years, it is more often the case where the amended North American system is introduced as a general method (Imaizumi 1970; Abe 1991b; Abe et al. 1994; Yoneda et al. 1996). Therefore, collectors who are planning to gather new measurement data, as well as any researchers who intend to donate voucher specimens to a museum collection, should bear in mind that field guides and textbooks published in Japan in recent years most likely follow the amended North American method in indicating the tail length and hind foot length without the claw (Hind Foot sine unguis: Hfsu) as explained above. However, there are very few countries other than Japan that have adopted the amended North American method as a national standard for measuring mammals; thus, one should be aware that the amended North American method is not regarded as an international standard. On the one hand, the British method is similar to the amended North American method in terms of hind foot length as it excludes the claw and tail length with its origin taken at the root of the tail vertebrae. On the other hand, the British method differs from the amended North American method in the way the tail is measured without bending it toward the back (Corbet and Ovenden 1980; Burton 1991). In China, the European method is adopted as a standard for measuring mammals (Wang and Ganyun 1983). In the case of ungulates, it is technically difficult to measure foot length exclusive of the hoof (homologous to claw), making the North American method the only way to measure hind foot length of ungulates. That also means the North American method is the only system that enables body measurements of terrestrial mammals, including ungulates, to be documented in a

consistent manner. Technical instructions for measuring small mammals following the amended North American method are provided below (cf. Abe 1991b).

Total Length (TL): Completely extend the body on its back and measure the linear distance from the tip of the snout to the end of the tail (excluding hairs at the tip of the tail). Loosen the muscles enough to stretch the carcass if it has undergone rigor mortis.

Length of Tail vertebrae (LTv): Bend the tail toward its back and hold it in an upright position against the body when laid out flat. Slide the edge of a ruler's short side backward along the lower back of the animal until it naturally stops at the root of the tail. Extend the tail straight along the long side of the ruler and read the scale at the terminal end of the tail (excluding hairs).

Head and Body length (HB): Take the difference of the TL value minus the LTv value above.

Hind foot length sine unguis (Hfsu): Stretch the hind foot toes well and measure the distance from the tip of the heel to the extremity of the longest toe (excluding the claw).

Ear length (E): Measure the length from the lowest edge of the intertragic notch, located between the tragus and the antitragus, to the furthest edge of the exterior front side of the auricle (excluding hairs).

Besides the basic measurements above, Fore Foot Length (FFL) and Fore Foot Width (FFW) are commonly measured for soricomorphs. FFL is measured from the rear end of the forefoot sole to the tip of the longest finger excluding the claw. FFW is the longest width across the forefoot sole. For chiropterans, Forearm length (FA), Tibia length (Tib), Tragus length (TR), and Wing Span (WS) are usually also measured. FA is the distance between the wrist joint and the elbow joint. Tib is measured from the knee joint to the distal end of the tibia. TR normally refers to the tragus length from the base to the tip along its inner edge. Please note that Handley (1988) defines two different types of

measurements for tragus: Total Length of the Tragus and the Length of the Tragus Blade. The former measures the tragus length along its outer edge, as opposed to the latter measuring the length along its inner edge. WS is the greatest width across the wings when the bat is in a position with both wings spread out. For large-sized animals, Height at the Shoulder (HS), chest girth, and other parts are measured. HS is measured straight from the upper edge of the shoulder blade to the bottom of the feet when the animal stands on its four feet. In Japan, Yoneda et al. (1996) introduced standard external measurements for cetaceans. The instructions in Yoneda et al. (1996) are almost the same as the standard measuring system for cetaceans endorsed by the American Society of Mammalogists. The only exception is that the body girth is measured at the umbilicus in Yoneda et al. (1996), whereas the American standard method records body girth at its largest. Follow Hamada (1986) for standard external measurements of the Japanese macaque. Most of the published manuals provide illustrations and figures in conjunction with instructions for recording external measurements of various groups of mammals (Nagorsen and Peterson 1980; Hamada 1986; Kitahara 1986; Abe 1991b; Geraci and Lounsbury 1993; Abe et al. 1994; Yoneda et al. 1996; Martin et al. 2001; Asano et al. 2006), so one should refer to those diagrams and instructions when taking animal body measurements until one becomes used to a set of metric data to be collected through specific methods of measuring each part of the body.

It should be noted that the morphometric values recorded by different investigators could often lead to significant biases in parameters calculated from external and skeletal measurements. Interobserver measurement error can be especially noticeable at body parts bearing significant individual variation or at those where the origin and/or the end point of a particular metric are indistinct and difficult to define (Palmeirim 1998; Blackwell et al. 2006). Therefore, as far as external measurements are concerned, it is recommended that a researcher take measurements of the same part at least twice, and use a mean value of variables for analysis (Blackwell et al. 2006).

4–5. Reproductive Condition Data

Observation of internal reproductive organs provides important biological data on the reproductive condition of an animal. Based on such data, one can understand

reproductive properties of a particular species in a specific region such as the yearly number of breeding seasons, length of a breeding period, litter size, number of parturitions per year, and age of reproductive maturity (Nagorsen and Peterson 1980).

Checking the size of the testes and visually inspecting the epididymal duct of caudal epididymis are commonly used as a simple method of determining the reproductive condition of males. For a fresh specimen that has not been dead long after its collection, measure the longest and shortest diameters of a testis after opening the abdominal cavity. When it is difficult to measure the accurate dimensions of a testis, including one preserved in fluid, at least make a note on the condition of the testes as to whether they have descended to fill the scrotum, in other words, scrotal (+), or non-scrotal / abdominal (-). For small-sized mammals, the reproductive condition of males can be determined by an inspection of the epididymal duct at the caudal epididymis: if the duct is visible, a positive indicator of sperm production and storage, the individual is reproductively active (+). Otherwise, mature sperms are not accumulating sufficiently in the testes, and, thus, the animal was reproductively inactive (-). In order to determine the reproductive condition of a male with a high degree of certainty, it is effective to prepare slides of a sperm smear or testis tissue for observation under the microscope. Whichever method is adopted, it is recommended to observe and document the reproductive condition of an animal immediately after its capture on a field catalogue or a field note.

As for the reproductive condition of females, it is obvious to tell that a pregnant or lactating animal is in a reproductively active (+) condition for certain, but it is also a reliable criterion to determine a female in active condition, or not by looking at the condition of the vaginal orifice, whether it is open (+) or closed (-). Additionally, inspect the condition of the uteri and document other reproductive criteria such as whether fetuses are present (+) or absent (-), and if placental scars present (+) or absent (-), as well as an external observation of the nipples, either developed (+) or not (-).

Methods of recording the reproductive condition of mammals as demonstrated above are discussed further by Imaizumi (1970), Nagorsen and Peterson (1980), Nakata (1986), and Abe (1991b), among others.

4–6. Collection Locality Data

A detailed and accurate description of the collection locality of specimens is a prerequisite for all studies pertaining to an emerging field of biodiversity research, not to mention conventional study with a primary focus on geographic variation and variation within a population of a species. For localities in Japan, it is required to record at least the prefecture, city, town or village, and a geographic feature such as a lake or a mountain. Furthermore, exact geographic coordinates of the point of collection should be recorded in latitude and longitude at least down to the minute. Latitude and longitude can be obtained with reference to the Geospatial Information Authority of Japan (GSI; formerly Geographical Survey Institute) standard base maps at a scale of 1:50,000 or 1:25,000. Alternatively, use of a car navigation system, or another device equipped with a Geographic Positioning System (GPS), if available in the field, enables a researcher to read latitude and longitude of a collection site on the spot by taking advantage of the GPS capability of such digital devices. Additionally, online topographic maps from the Map Browsing Service (http://watchizu.gsi.go.jp) published by GSI are also of use. It is further recommended to record a collection locality in reference to a 3rd Mesh code number assigned to each 1km× 1 km land grid on a map of Japan developed for the National Survey on the Natural Environment (Japanese Environment Agency 1997). For countries outside of Japan, it is desirable to document a place name as an administrative unit as well as its latitude and longitude according to the standard recording system in Japan as described above. Ideally, it is best to map points of collection on a relatively large-scale contour map (1:50,000 to 1:200,000) attached to a field catalogue or notebook.

4–7. Recording Additional Data

It is good to record habitat features at collection localities, as it provides an indication of the ecological distribution of a particular species. Useful habitat data that should be recorded in the field include the landscape, dominant vegetation, elevation, condition of soil and other related information. The method of collection (e.g., trapping, netting, salvage of carcasses) also should be recorded, but not necessarily for each individual specimen when all the specimens were obtained using the same method in one field session. If that is the case, it is sufficient to summarize the specimen collection method at the top of the first entry of a session in a field catalogue or notebook, making it

unnecessary to repeat the same information for the remaining data entries. Other behavioral and ecological observations can also be recorded as supplementary data in a field catalogue or notebook. Specific examples include the time of specimen capture, unusual color phases (e.g., partial albinism, whitening on the tip of the tail), climatic conditions at the time of collection, vocal communication, and any field signs found left behind. It may seem cumbersome to make notes of miscellaneous information, but such data often turns out to be an invaluable reference for natural history studies in the years to come. Photographs of a collection site and the animals collected are particularly valuable for extremely rare species in the wild or for an individual showing peculiar pelage variation. Close-up photography is an effective means of documenting the facial region of a live-captured mammal, including its facial parts that may shrink and no longer be recovered to their original forms once the animal is preserved as a specimen (Nagorsen and Peterson 1980).

5. Preparation of Mammal Specimens

Generally, mammal specimens that are intended to be preserved in a collection room are classified into three different categories determined by different types of preparation: 1) study skin and skull (which may include a partial post-cranial skeleton); 2) complete skeleton including skull; 3) fluid-preserved whole body specimen. Each preservation type presents strengths and limitations, and the choice of preparation should be made based on a particular research purpose.

5–1. Preparation of Fluid-Preserved Specimens

This is a method to pickle and preserve a whole animal carcass in 70% ethyl alcohol or 10% formalin solution (i.e., 4% formaldehyde aquatic solution) sealed in an airtight container. It is necessary to take body measurements of a specimen before it is placed in fixative because the chemical process of fixation causes hardening and shrinkage of the body. It is recommended to make a partial ventral incision in each specimen to facilitate infiltration of the fixative in to the body cavity (Abe 1991b). Formalin solution is stronger than alcohol for effective specimen fixation, but is known to break down into formic acid over time (in a matter of 1-3 years) leading to the

decalcification of teeth and bone tissue of specimens in the solution. In order to prevent this undesirable degradation, specimens need to be transferred from the original 10% formalin or other fixative solution after fixation, to 65–70% ethyl alcohol or 45–60% isopropyl alcohol for permanent preservation (Nagorsen and Peterson 1980). If it is difficult to switch the solutions, there is a way to neutralize the solution by adding ammonia solution, marble fragments, or hexamethylenetetramine (Hayashi 1981). On the other hand, Bouin's fixative solution requires special caution when used, because its main chemical component, picric acid, is explosive. Specimens that are fixed and preserved directly in ethyl alcohol solution, instead of being fixed initially with formalin, makes it possible to subsequently obtain a tissue sample from such specimens for DNA analysis. Whereas the method of directly preserving specimens in alcohol of exactly or almost 100% concentration is deemed optimal for preparing DNA samples, the compromise is that it may cause shrinkage of the musculature, which results in deformation of the skeleton. For this reason, it is better to take and preserve DNA samples, such as minced liver tissue, separately at the time of preparing fluid-preserved specimens. As for specimen storage jars, a large, colorless, transparent glass container (a wide-mouth container with airtight sealing like the one typically designed to brew plum wine) or a smaller glass container with a cap lined with an inner closure (usually referred to as a "mayonnaise jar," available at scientific laboratory equipment and supply vendors) is generally recommended. On the other hand, recycled food containers, such as emptied powder coffee bottles, are inadequate and should never be used for storing specimens because of their non-airtight sealing, which causes evaporation of fixative solution as well as their metal lids, which are prone to rust. Labels associated with fluid-preserved specimens should always be soaked in solution together with the specimens. Motomura (2009) discusses in detail the techniques of preparation, management, and photography of fluid-preserved fish specimens. For the most part, the information therein is also applicable to mammal specimens preserved in fluid.

5–2. Preparation of Skins

Standard techniques for preparing museum skins are discussed in the literature (e.g., Abe 1991b). Medium to large-sized mammal specimens are often preserved dry, as

laid out hides or tanned skins for scientific use. On the other hand, for small-sized mammals, there are three different types of preservation methods:

1) Flat skins: The skin is cut open along the ventral median line. Once removed from the body, the inner surface of the skin is coated with a preservative that prevents hairs from falling off (e.g., borax powder or dried alum, or a mixture of equal parts of camphor and either borax or alum), then the skin is stretched flat on a board, pinned, and left to dry.

2) Study skins: The skin is incised longitudinally on the posterior abdominal midline, but not all the way anteriorly. Next, the skin is peeled off the body and cleaned by removing fat and muscle from its interior. After a dry preservative such as those described above is applied to the inner side, the skin is stuffed with cotton, to achieve just the right level of hardness. The stuffed skin is then sewn up with a cotton thread to close its openings. The skin is pulled into its natural shape, secured on a flat board for drying, and pinned at the four feet. It is effective to replace the tail vertebrae and all four of its skeletal legs with wire, bamboo rods, or gramineous grass rachises so as to keep the skin in shape and prevent breakage.

3) Flat skins of small mammals mounted on cardboard: The ventral side of the skin is cut open transversely across the hind legs, and the hind leg bones are cut off without damaging the skin. Finally, the whole skin is removed from the body. The interior of the skin is treated with a dry preservative as mentioned above. The skin is stretched over a paper body (cardboard; acid-free paper is recommended to avoid deterioration of the skin through contact), and the hind legs and tail are stapled onto the same cardboard with thread or staples made of stainless steel. The size of a paper body is based on the actual body size of the animal. The cardboard also serves as a specimen label for writing field data and measurements.

Various manuals have been published that illustrate, in detail, step-by-step techniques of preparing mammal skins along with diagrams (British Museum 1968; Imaizumi 1970; Hashimoto 1979; Nagorsen and Peterson 1980; Pucek 1981; Honda

1985; Abe 1991b; Martin et al. 2001). One should refer to these references often until becoming accustomed to preparing skins.

5–3. Preparation of Skulls and Skeletons

Once the skin is prepared following one of the methods illustrated in 5–2, one should promptly prepare the skull. Cut the skull off from the skinned body at the joint of the first vertebra (atlas), but care should be taken not to break the occipital region of the skull during the procedure. On the one hand, for relatively small mammals (soricomorphs, murids, and bats) with fragile skulls that can easily be damaged, it is recommended to dry the skulls without removing flesh. On the other hand, for animals larger than a squirrel, it is better to remove the brain, eyeballs, tongue, and thicker layers of muscle from the skulls before drying. Skulls that are separated and dried in this manner during fieldwork should be labeled immediately with the same number as that assigned to and recorded on the specimen label attached to the matching skin before they are brought back to the lab, along with the rest of the specimens upon completing fieldwork. If these dry skulls are not immediately ready to be cleaned afterwards, they can be kept dry in insect-, dust-, and light-proof containers for the time being. One of the easiest and most effective means of cleaning a skull is to boil it in hot water to sufficiently loosen up muscle and soft tissue attached to the skull. The softened tissue can then be removed by hand with a pair of forceps and brain tissue can be scooped out of the braincase through the foramen magnum using various tools such as forceps, pins, and nails. If the facility is equipped with a dermestid beetle colony, the larvae and adults can also be used to effectively to clean the skulls. When cleaning skulls of medium- and large-sized mammals, the following procedure is recommended to prepare quality specimens: pretreat the skull by boiling it in hot water and removing most of the flesh, then soak the skull in an aquatic solution of proteinase overnight to break down the soft tissue further; at last, wash the skull to clean it of soft tissue (Hachiya and Ohtaishi 1994). The enzyme brand known as Tasinase N-11-100, which Hachiya and Ohtaishi (1994) originally recommended, is no longer available. As a substitute, bioprase AL-15-FG (manufactured by Nagase ChemteX Co.) can be used at an optimum temperature of about 50°C, which is achieved by the use of a water heater with a thermostat. For large-sized mammals, aerobic bacteria can be

also used instead of enzyme. Create a maceration bath heated to 37–38°C with constant aeration of small air bubbles pumped into the water. After soaking under these conditions for a few days, the skull would be completely cleaned of soft tissue by the bacteria proliferating in the system. The use of enzyme or aerobic bacteria requires good ventilation as it generates a strong odor during processing. The use of caustic chemicals, including bleach, pipe cleaning products, and strong alkaline agents (e.g., sodium hydroxide, potassium hydroxide), should not be readily used as a way of removing flesh from a skull. These substances are known to cause destruction and deformation of bone as well as delamination of tooth enamel.

Whichever cleaning method is used, the defleshed skull may be bleached for aesthetic purposes. Soak small mammal skulls overnight, or for medium- to large-sized mammals, soak for 3–5 days in approximately 10% hydrogen peroxide solution. Then, wash the skulls thoroughly with water and allow them to air-dry. However, it is unnecessary to bleach specimens prepared specifically for research or museum preservation. Bleaching is also usually unnecessary when the skull is cleaned with aerobic bacteria.

Preparation of the skeleton is conducted as follows: skin the specimen using one of the methods described in 5–2, and cut open the abdomen to obtain data about its reproductive condition (see 4–5). Next, remove all internal organs from the abdominal and thoracic cavities (the remnant viscera can be a cause of decomposition). Depending on the purpose of study, various samples are taken from the tissue and organs at this stage. Subsequently, remove larger chunks of muscle from the remaining body and let the skeletal material dry. The method of preparing a skeletal specimen from the dry body is basically the same as that used for the skull (although the larger the animal's body mass, the more difficult it becomes to process). Refer to Hachiya and Ohtaishi (1994) if degreasing is needed to prepare skulls and skeletons. Ammonia water, which is safer to use than hydrogen peroxide solution, is effective in degreasing and lightly bleaching skeletal material. However, if articulation, sutures, minute bones, and cartilage need to be preserved intact, keep chemical treatment, like bleaching and degreasing, to the necessary minimum.

6. Transport and Management of Mammal Specimens

6–1. Transportation Methods for Specimens and Data

The transportation methods demonstrated below are mainly those recommended by Nagorsen and Peterson (1980) on the assumption that specimens and their data are going to be transported from a field collection site to a museum facility. Situations differ significantly from one case to another, for example, when specimens are carried by collectors themselves, as opposed to when they are shipped by a third party such as the mailing service. There are also considerable differences between domestic and overseas transport. Nonetheless, the following basic points apply to most of these various modes of transportation. The methods recommended herein should also be applicable in cases where completed specimens are moved between museums, among individual researchers, as well as between museums and researchers.

Fluid-preserved specimens: A wet specimen properly fixed in formalin can be kept in a moist condition during its transport by adding a very small amount of 10% formalin solution in a tightly sealed packing container. The same approach can be taken for specimens kept in a fixative or preservative other than formalin, but the key point is to use basically the same kind and strength of fixative or preservative as those solutions the specimens are already fixed and preserved in. It is necessary to pack wet specimens along with cotton, cheesecloth, or newspaper in each container to fill the gap and prevent the specimens from shaking, and at the same time, to keep moisture in the enclosure. Cheesecloth is superior as filling, as it can cover the entire surface of a specimen and keep it moistened effectively. When selecting a packing container for specimen transport, a wide-mouth plastic jar is ideal. If that is not available, a tightly sealed plastic bag is an acceptable substitute. On the other hand, a glass container is inappropriate for specimen transport. If specimens need to be transported in a glass jar, the whole container must be packaged in a sturdy box together with enough cushioning material to fill the gap and prevent the glass from breaking. If plastic bags are used for packing, put the specimens with cotton and fixative solution in an inner bag, and seal them tightly after removing air from the bag. Next, seal the bag in another plastic bag, and place the double-bagged content in a metal box (such as an emptied commercial cake box), with a matching lid

firmly secured in place with adhesive tape. If there is a large gap between the metal container and the bagged content, make sure to fill it with cushioning and absorbent materials such as cotton, cheesecloth, and used newspaper. The whole package wrapped in this manner is usually compact, and as far as it is handled with care to prevent leakage of the fixative solution, it can be shipped by regular parcel post or other delivery services domestically. On the other hand, overseas transport requires different procedures according to the policies and regulations of each specific country and region as well as the present situation at each destination. For example, use of natural cotton in a package may become a problem upon quarantine in some area, so attention should be paid to using only synthetic cotton instead. Additionally, with regard to air parcels, the chemical class and quantity of solution contained in each package could be subject to strict regulations.

Skins and skulls: It is ideal to transport skins in a container made of sturdy plywood, but it is also acceptable to use a corrugated cardboard box for specimen shipping purposes. First, line the bottom of a container with a sheet of cotton or similar cushioning material as thick as about 5 centimeters deep. Evenly spread several dry study skins above this bottom cotton layer. Line another flat layer of cotton on top of the specimens for more skins to rest on. Repeat this layering until the container becomes full to its top edge with the uppermost layer of cotton added to about 5 cm deep.

If skulls are completely dry and pest-free with no chance of infestation with insects or their eggs, they can be shipped in the same container with dry skins. Otherwise, skulls must be shipped separately in a different sturdy container. If it is expected to take several days or longer to transport skins, it may be necessary to treat the specimens by adding a paradichlorobenzene-based insecticide, commercially known as parazol, in the same shipping container to repel moths and skin beetles, and prevent their eggs from being laid on skins. Alternatively, if ever possible, pretreat specimens to eradicate pests by other non-chemical means such as by subjecting them to cold temperatures before packaging them for transport. If the use of pesticides is necessary for a shipment of skins, skulls should not be sent together with such a package because many museums use dermestid beetles for skull and skeletal cleaning, and the presence of insecticide on the skulls may inhibit the beetles' defleshing activity.

Skeletons: Skeletons must dry out prior to being enclosed in a shipment. Skeletons of small-sized mammals can be transported together with matching skins in the same container as long as insect repellents are not enclosed in the shipment. If that is the case, skeletons should be packed in the bottom of a container first. Next, a layer of cotton is placed on top of the skeletons and, finally, skins should be placed evenly on the top layer. In contrast, heavy skeletons from large-sized mammals need to be transported separately in a sturdy wood-framed crate. If such large-sized skeletons are dehydrated, it is recommended to wrap them with plastic bags just before transport to reduce the strength of their odor. If skeletons of extra-large mammals are to be shipped, the receiving museum should be notified ahead of time.

Data: Original copies of field catalogues and notebooks, as well as topographic maps associated with specimens collected in the field should be mailed separately from the specimens themselves by express mail or airmail. In case they are mailed overseas from a country that has an unreliable mailing system, the items should be shipped by registered mail.

6–2. Regulations on Importation and Exportation

The Washington Convention, or officially the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), originally came into effect in 1975. It has been applied to trade in Japan since November 1980. Under this treaty, without legal permits, both imports and exports are banned for endangered and threatened species that need special protection. This regulation applies to all international transactions, and as long as a species is listed as threatened, not only a whole individual body (whether live or dead), but also the parts thereof (including skulls, skeletons, tissue, cells, and biochemical samples) and their artifacts (including taxidermy) are subject to restrictions. As long as either one of the countries of international trade is a CITES Party, all wildlife trade is subject to strict monitoring as to whether the necessary permits have been acquired. Species under the CITES regulations in international trade are listed under three different categories in the Appendices. Species listed in Appendix I are the most endangered species. Species listed in Appendix II are species that are not necessarily threatened with extinction today, but whose trade requires close monitoring in order to

prevent them from becoming endangered. Species listed under Appendix III are species for which either one of the CITES Parties recognizes a need for regulating trade within their jurisdiction. Permits are required from both the importing and exporting countries for international trade in the species listed in Appendix I. All species in Appendix I, as well as domestic species designated as national endangered species of wild fauna and flora, including 4 species of mammals (i.e., Tsushima leopard cat *Prionailurus bengalensis euptilurus*; Iriomote leopard cat *Prionailurus bengalensis iriomotensis*; Daito flying fox *Pteropus dasymallus daitoensis*; and Amami rabbit *Pentalagus furnessi* [recently, Bonin flying fox *Pteropus pselaphon* was added]), are restricted for domestic transport (with mandatory applications and permits). Legal procedures are handled differently on a case-by-case basis, for example, a transaction between museums versus between a private party and a university. Therefore, it is necessary to consult with the Wildlife Division, Nature Conservation Bureau of the Ministry of the Environment before transporting these species. See the Environmental Agency Wildlife Conservation Administration Study Group (1995) and other references for details. As for the species listed in Appendix II, an export permit from the exporting country is required for shipping them overseas. As for the species listed in Appendix III, international trade requires an export permit from the exporting country only if that country lists this particular species in Appendix III, in addition to a certificate of origin. Any party involved in wildlife trade must be well versed in the details of actions regulated in Japan pertaining to CITES and the list of species in Appendices through the Wildlife Conservation Issues Study Group (1988) and so on. Note that the CITES Conference of the Parties holds a meeting approximately every two and a half years, in which amendments to Appendix I and II are discussed and adopted (Kaneko 2001). Thus, it is important to obtain the most up-to-date information in order to ensure that the particular species of specimens that you intend to transport does not infringe on these regulations. Refer to Kaneko (2001) regarding wildlife import clearance procedures following the Washington Convention, a list of CITES Management Authorities and Scientific Authorities in Japan, as well as a list of Management Authorities of the ASEAN (= Association of Southeast Asian Nations) Parties. If there is anything unclear about the formalities concerning CITES, you should consult with the appropriate Scientific

Authorities and Management Authorities (for example, for terrestrial animals, contact the Wildlife Division, Nature Conservation Bureau, Ministry of the Environment, and the Trade Licensing Division, Trade and Economic Cooperation Bureau, the Ministry of Economy, Trade and Industry) (Kaneko 2001). On top of CITES, be aware of the domestic regulations by the Wildlife Protection Law, Section 20, Paragraph 2 [currently, WPML, Sections 25, 26 and 27], which control the export and import of individual mammals and their products (see Wildlife Protection and Management Study Group 2001 for further discussion). Upon conducting fieldwork overseas, the local country's laws and regulations, as well as international treaties, must be followed in addition to CITES (Ad hoc Committee for Animal Care Guidelines 1985).

6–3. Collection Management

The management of a systematic collection at a museum encompasses a wide range of activities from the accessioning of specimens to the preservation treatment necessary to prevent the deterioration of specimens in the collection (Wiley 1981). Examples of published works in the form of field collection records, specimen catalogues, or taxonomic reviews available in Japan are Maeda (1984, 1986), the Second Department of Anatomy, School of Dentistry, Aichi Gakuin University (1985, 1986), Miyazaki (1986), Shigehara (1986), Tomida and Sakura (1988, 1991), Yoshiyuki (1989), The First Department of Anatomy, School of Medicine, Dokkyo Medical University (1992), Endo (1996, 1997, 1998, 2000), Endo et al. (2001, 2002), Zholnerovskaya and Koyasu (1997), and Yoshiyuki and Endo (2003). However, remarks on these publications are beyond the main scope of these guidelines. The Systematic Collections Committee of the American Society of Mammalogists (2004) established "basic curatorial standards for systematic collections of mammals" which are included in an appendix to these guidelines [The appendix was deleted in this English version]. These standards, as well as Wiley (1981), are useful references for those interested in learning more about museum collection management.

7. Public Health

Every individual mammal in the wild should be viewed as a potential carrier of some sort of zoonotic disease. Similar to those typically known as endemic, many of the diseases occur locally, so it is not realistic to make exhaustive lists of possible zoonoses that could be transmitted during the process of collecting and preparing mammal specimens (In Japan, a general list of zoonoses is found in Kamiyama (2004) and Kimura and Kida (2004), whereas Yokohata (2009) covers diseases specifically transmitted via wild mammals). Therefore, when researchers are ready to conduct field collecting, they should be concerned with any potential diseases that can be contracted from wildlife in the study area, and educate themselves beforehand to learn more about them. If there is a risk of becoming infected with a disease, such as typhus or rabies from captured animals during field activities in areas outside of Japan, it is necessary to consult a medical doctor about options for vaccination against such diseases. Caution is advised, even in Japan, as multiple zoonotic diseases are known to potentially infect researchers directly or indirectly, through native animals such as echinococcosis, tsutsugamushi disease (scrub typhus), Lyme disease, hemorrhagic fever with renal syndrome, and Japanese spotted fever (e.g., Suzuki and Ikeda 1985; Arikawa and Hashimoto 1986; Arikawa 1996; Takahashi 1998; Asano et al. 2006). Furthermore, in recent years these contagious diseases, emerging infectious diseases in particular, are viewed as a threat not only to humans and livestock but also to biodiversity (Daszak et al. 2000). It is also important for researchers to act carefully so as to not serve as a source of infection to other people, livestock, and wildlife as a transmitter of a pathogen.

When handling live mammals, your hands need to be protected with thick cotton or leather gloves, and you must pay attention not to be bitten. Fortunately, the chance of rabies infection from wild animals in Japan is considered to be minimal. In contrast, outside the country, mammals that are well known potential vectors of rabies include bats, foxes, mongooses, and skunks, just to name a few. Comparatively safer circumstances in Japan might contribute to the fact that most Japanese researchers tend to take fewer safety precautions against the risk of rabies. Therefore, if it is necessary to catch animals for fieldwork overseas, as long as the occurrence of rabies has been reported from the particular country or region, it is important to handle the animals carefully on the presumption that every animal caught in the field is potentially infected with rabies. It is

most desirable to receive a rabies shot before conducting fieldwork in any country or region with a high risk of rabies, even if vaccination is not a mandatory part of the country's entry requirements. Including information on vaccination, each field researcher must be equipped with sufficient knowledge of personal healthcare overseas prior to their departure (see Miyazaki 1999). Refer to Constantine (1988) about health risks and necessary handling measures, specifically pertaining to bat research.

When preparing mammal specimens, common sense precautions can help reduce the risk of infection. It is recommended to wear latex gloves during dissections, and it is even better to use a disposable paper mask to stay on the safe side. Animal excreta must not be touched by bare hands, as this can be a source of infection. Dissection utensils should be disinfected using appropriate antiseptic after each use. The handling of roadkill also requires precautions against possible infection. If you notice the following medical symptoms subsequent to specimen collection or preparation, you must consult a medical doctor and report a possible zoonotic infection to obtain an accurate diagnosis: a flu-like symptom, a chronic respiratory problem, an enlarged lymph node, or signs of high fever, and vomiting or diarrhea. Incidentally, the Wild Animal Medical Center, Rakuno Gakuen University, Hokkaido, and the Laboratory of Veterinary Pathology, Faculty of Applied Biological Sciences, Gifu University, Gifu, serve as hubs of zoonoses research involving wild animals in Japan. These institutions accept animal remains that are suspected of being potential sources of infection.

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