Guidelines for the Procedure of Obtaining Mammal Specimens as Approved by the Mammal Society of Japan (2023 Revision)

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

Wildlife research communities are confronted with complex challenges encompassing issues related to nature conservation and animal welfare. These research communities also face various societal concerns pertaining to their responsibility to produce credible scientific studies and adhering to professional criteria throughout the publication process. Numerous academic societies and related organizations whose members conduct scientific studies involving animals have established policies and guidelines pertaining to the handling of live animals and preserved animal specimens (Japanese Association for Laboratory Animal Science 1991; Japanese Society of Zoo and Wildlife Medicine 2010; Nature Conservation Committee and Editorial Committee of the Ichthyological Society of Japan 2004; Japan Ethological Society 2005; Science Council of Japan 2006; Primate Society of Japan 2013). Within the Mammal Society of Japan (MSJ), the Committee of Reviewing Taxon Names and Specimen Collections (hereinafter referred to as the "Committee") instituted the first edition of the Guidelines for the Procedure of Obtaining Mammal Specimens (hereinafter referred to as the "Guidelines") under the leadership of Dr. Kazuhiro Koyasu in 2001 (Committee of Reviewing Taxon Names and Specimen Collections 2001; in Japanese). The first paragraph of the first edition of the Guidelines explains in detail the background information that led to their establishment. The MSJ has since seen increasing nationwide interest in environmental issues, animal welfare, and zoonotic diseases. In addition to this overall trend, major revisions to existing wildlife regulations and the enactment of new domestic legislation directly affecting wild mammal studies in Japan, such as the Wildlife Protection and Hunting Law and the Invasive Alien Species Act, have prompted the MSJ to acknowledge the need for timely revision of the Guidelines. Therefore, the Guidelines Revision Task Force was established under the Committee in 2006, and a revised version was approved by the MSJ council in July 2009 (Committee of Reviewing Taxon Names

and Specimen Collections 2009). The contents were reviewed in 2015, and the annotated guidelines were finally published on the MSJ homepage (https://www.mammalogy.jp/en/guideline.pdf).

The purpose of the Guidelines is to provide professional standards for the evaluation of specimen handling procedures in mammalogical research, including field collection methods, with the endorsement of the MSJ. Given that the capture of wild mammals in the field normally precedes specimen preparation, the Guidelines address not only field research techniques but also ethical considerations from the standpoints of animal welfare and conservation. The MSJ has not yet adopted procedural guidelines for the handling of live animals; therefore, similar guidelines developed by other organizations should be referred to where appropriate (e.g., Japanese Association for Laboratory Animal Science 1991; Fukui 1991; Japanese Society of Zoo and Wildlife Medicine 2010; 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; Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). Ethical aspects of conducting research using live animals have also been outlined by 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 decisions regarding which project design and method to adopt. The intended users of the Guidelines are mainly MSJ members and other non-member mammalogists. However, we expect that the Guidelines will also be of interest to a broader audience, such as natural history museums with no mammal expert staff, policymakers and government agencies responsible for legal regulations related to mammals, foundations and support organizations that provide research grants to mammalogists, and editors and reviewers of academic journals that accept papers in the field of mammal research.

1. Value of Specimens in Mammalogy

The field of mammalogy encompasses a broad range of research hypotheses that

are tested through various approaches. In studies involving wild mammals, it is frequently necessary to capture animals live or euthanize them to acquire essential data from the study material. The information thus obtained enables accurate species identification and provides an understanding of numerous scientifically important topics, including 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, and the ecology of mammals in their natural or managed environments (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987). Among mammalogical societies outside Japan, 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) as vouchers after completion of the study. A collection in a museum that meets such standards can be used as a permanent repository of voucher specimens and their associated data examined by different researchers. Voucher specimens preserved in this manner constitute a systematic collection that enables validation of past research and represents a resource for present and future research. Abe (1992a) provided a detailed discussion of the significance of voucher specimens collected and preserved in mammalian research from the standpoints of taxonomic information, ecological and physiological information, and other ancillary information.

Maximizing the scientific value of specimens requires adequate preparation, documentation, and preservation of original specimens and their associated data. The Guidelines aim to provide professional benchmarks by which to standardize the procedures of collecting and preparing mammal specimens and documenting associated data. The Guidelines are also designed to function as a practical guide for mammalogists involved in specimen preparation. Moreover, discussions on the professional ethics and techniques related to wild mammal collections are generally pertinent to ecologists.

2. General Issues Concerning Mammal Collection in the Field

Sampling protocols for mammal specimens include not only traditional lethal specimen collection for museums, but also nonlethal approaches such as biopsy

sampling from live-captured animals, followed by animal release. The Guidelines mainly consider the ramifications of adopting lethal methods. Hamazaki (1988), Kishimoto (2002), and Kaneko and Kishimoto (2004) pointed out animal and worker safety and minimal environmental impact as the "three basic rules" of animal collection, placing emphasis on the need for compliance, significance of the rules, and other points of consideration. Although initially proposed with a focus on nonlethal methods, these three principles are also relevant to lethal methods. Consultation of the Handbook of Japanese Wildlife Care and Medicine Editorial Committee (1996) and Bookhout (1996) is recommended for technical guidance on aspects of stabilization, rescue, and external measurement of data collected from live-captured wild mammals.

2-1. Laws on Field Collection

The following discussion is based on a report by Ikeda and Hanai (1988), who published one of the first review articles on the regulations and legal procedures relevant to field mammal collectors in Japan. The topic is divided into three main sections: the Wildlife Protection and Hunting Law, more commonly referred to as the Wildlife Protection Management Law (WPML) (original Japanese title: *Choju no Hogo Oyobi Kanri Narabini Shuryo no Tekiseika ni Kansuru Houritsu*); the Law for the Protection of Cultural Properties (*Bunkazai Hogo-ho*); and other relevant laws. The WPML underwent 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 facilitated a broader and more inclusive application of the existing law to all Japanese mammals, encompassing groups that were previously excluded from its scope: order Eulipotyphla, family Muridae (excluding pests caught by agricultural and forestry workers, and three residential species: *Mus musculus*, *Rattus rattus*, and *Rattus norvegicus*), and certain marine mammal taxa (i.e., dugongs and certain pinnipeds; see below for other marine mammals that remain exempt from said regulation) (Ishinazaka 2003; Yokohata 2003). To collect any wild mammal species regulated by the WPML for research purposes, either one of the following two types of collection permits is required: a Wildlife Collection Permit issued under WPML Clause 1, Article 9 or a Class A Hunting License issued under WPML Article 39. The

latter is more restrictive than the former in that the permit holder is allowed to capture only those species listed as game animals using regulated tools and equipment within a restricted hunting zone for a limited period of the hunting season.

Next, according to Article 125 of the Cultural Properties Protection Act, permission from the Commissioner for Cultural Affairs is required to "change the current status" of natural monuments and special natural monuments. This change includes not only the collection of animals but also their release after research samples are obtained. Applications for such permission must be submitted to the Agency for Cultural Affairs through the department responsible for cultural properties at the prefectural or municipal level, making it advisable to first consult with these departments. Notably, it usually takes about 2 months to receive permission after applying.

Marine mammals are regulated by the Fisheries Act, the Fisheries Resources Protection Act, and the Sea Otters and Fur Seals Hunting Control Act. In particular, whales are subject to the Act for Ensuring Sustainable Use of Whales. In addition to these resources, the following are useful for 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). The appropriate governmental agencies responsible for issuing collection permits should be consulted for further details. Hatakeyama (2004), Sakaguchi (2007), and the Nature Conservation Bureau of the Ministry of the Environment (2017) have added to the literature on this subject. Because this information is continuously reviewed, the website of the Ministry of the Environment should be always checked for the latest information (https://www.env.go.jp/nature/choju/index.html).

Alongside these general laws, the collection of Japanese mammals listed as threatened species is now regulated under the Endangered Species of Wild Fauna and Flora Conservation Law, issued in 1992 and enforced since April 1, 1993 (Environmental Agency Wildlife Conservation Administration Study Group 1993, 1995; Management and Coordination Agency Administrative Evaluation 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 Section 6-2.

In summary, the collection, import, and/or export of wild mammals are subject to domestic and international regulations based on wildlife laws and treaties, such as those described above. Therefore, if a researcher's fieldwork involves species, locations, or activities covered by these laws, acquiring valid permits issued by authorized agencies is mandatory. These laws and acts may undergo amendments with the future inclusion of new regulations. Therefore, each researcher is accountable for becoming familiar with all regulations that impact their study area and obtaining 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. To legally collect these animals for scientific study, a researcher must obtain permission from the Minister of Agriculture, Forestry, and Fisheries based on either the Enforcement Regulations of the Fisheries Act, Article 1 or the Enforcement Regulations of the Fisheries Resource Protection Law, Article 1. Likewise, the collection of domestic sea otters and fur seals is largely prohibited by the Sea Otters and Fur Seals Hunting Control Act. For scientific collection of these groups of Japanese sea mammals, researchers must obtain a license from the Minister of Agriculture, Forestry, and Fisheries in accordance with Article 1 of the Enforcement Regulations of the above law.

In addition to acquiring field collection permits, researchers may also need to obtain approval related to public and private land use. For example, the submission of a forest entry notification from a district forest office is required to enter a national forest, and permission from a landowner and land manager is required before wildlife is collected on public land. Furthermore, the Invasive Alien Species Act (issued in 2004 and enforced since October 1, 2005) prohibits the rearing, possession, and transfer of living animals that are considered invasive alien species, including 22 taxa and 2 hybrids of mammals (as of 2022), without permission. General guidance on biological fieldwork and relevant laws is provided by Komaru (2001), the Nature Conservation Society of Japan (2010), and Kohyama (2018). Inquiries about the field

sampling of organisms should be directed toward the websites of relevant ministries and agencies as well as local governments.

In addition to live capture, animals killed through events such as traffic accidents can be obtained as specimens. This is an efficient collection method considering the numerous legal restrictions on animal collection. Because many wild animals that die in traffic accidents are collected and disposed of by offices of local governments, in some cases animals are collected after consultation and subsequently stored in a freezer (Takatsuki and Tatewaki 2010). When requesting specimen preparation by a taxidermy company, it may be necessary to certify that the item was not obtained illegally. Some local governments issue notifications of found birds and animals; thus, it is recommended to contact the department in charge. Species designated as natural monuments or special natural monuments are subject to the Cultural Properties Protection Law, whereas dead animals and specimens are not. However, when discovering and preserving a deceased animal as a specimen, it is advisable to follow the procedures outlined by the Agency for Cultural Affairs. We recommend consulting with the department responsible for cultural properties in the specific jurisdiction and submitting a notification of loss (*messitsu todoke*). When collecting cetacean specimens that have been stranded on the coast, it is necessary to submit a report on the processing of the cetacean carcass and a notification of possession for academic purposes in accordance with the requirements of the Ministerial Ordinance on the Permission, Regulation, Etc. of Designated Fisheries. Furthermore, for blue whales, bowhead whales, gray whales, and porpoises, it is essential to obtain permission from the Minister of Agriculture, Forestry, and Fisheries. For baleen whales and sperm whales, DNA analysis results must be reported for individual identification.

2-2. Ethical Issues Related to Field Collection

Ethical issues regarding wildlife sampling were clearly outlined by Nagorsen and Peterson (1980). The possession of a valid collection permit does not give a collector the right to collect animals irresponsibly. Fieldwork should be conducted using the most humane method possible, while striving to minimize the impact on the local biota without disturbing sampling sites. Indiscriminate mass collection must not

be performed, particularly in circumstances where numerous animals are clustered in one area. Mammals should be captured live if possible, and as soon as the required sample size has been reached, any surplus live animals should be released unharmed. Such flexibility is possible only when utilizing live traps; there is no corresponding option for researchers employing kill traps. Notably, the required sample size for a particular study is contingent on the study design.

Therefore, every investigator who conducts fieldwork is responsible for determining the specific sample size required for their study, and it is imperative that they collect no more specimens than necessary (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987; Animal Care and Use Committee 1998; Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). Conversely, sampling too few animals may hinder the investigator from achieving the study objectives and thus lead to the wasteful use of animals (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016).

Friend et al. (1994) convincingly justified the need to adopt lethal methods in wildlife studies as follows: "[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." Friend et al. (1994) further established five criteria for assessing a proposed study requiring animal collection: the proposed collection will provide scientific data that is not duplicative of information already in the scientific literature or presently available in accessible scientific collections and repositories, suitable information cannot be obtained from alternative methods that do not require taking live animals, the methods of collection are acceptable and effectively minimize the potential of trapping non-target species, the target animals can be killed quickly and cleanly, and appropriate methods are employed to be as age- and class-specific as possible according to the purpose of the study. Specimens intended to be preserved in a museum for the establishment of a systematic collection must be prepared with meticulous care, and scientific data associated with the specimens must be documented and archived using a standard method (see Sections 4-1 through 4-7).

3. Animal Collection Equipment and Techniques

3-1. Collection Using Kill Traps and Guns

When collecting mammals using kill traps, special attention should be paid to avoid unnecessary suffering and damage to body parts that must be preserved for the study. The all-plastic snap trap commercially known as a "Panchu trap" in Japan is one of the most convenient and easiest traps to use for catching small mammals such as rodents and terrestrial insectivores (Abe 1991a, 1992b; Murakami 1992; Yoneda et al. 1996). Other types of snap traps include the Victor brand trap and various other metal traps; however, these models [excluding the Museum Special (made in the United States) and a custom-built metal snap trap (see Murakami 1992)] are prone to crushing skulls (Imaizumi 1970). Therefore, slight adjustments to the device may be necessary, such as repositioning the bait trigger plate. Murakami (1992) provided source information about these products. Various types of mole traps have been introduced, including the scissor trap, harpoon trap, and a tube trap with a wire loop for strangling (Abe 1992b; Yokohata 1998). Traps for water shrews include a large snap trap with a bait trigger plate, a tube trap attached to a mesh screen, and a "*mujiri*" cage traditionally woven from reeds or bamboo (Abe 1992b). Water shrews may also be caught by setting common cage traps in streams (Obara 1999). Using these kill traps is considered an adequate means of small animal collection that aligns with both research objectives and ethical standards.

Decisions regarding the trapping method and trap positioning should be based on maximizing the odds of catching the intended species, while minimizing the chance of trapping non-target animals. In particular, kill traps should be avoided in areas where endangered species may be collected. To avoid overlooking traps, it is important to mark each trap station with a marker such as colored tape. In addition, each trap must contain a label that includes information such as the permit holder's name, the name of the office issuing the permit, and the permit ID number. If a trap is too small to label, a placard or sign must be posted at the site. All traps must be checked at least once daily, ideally early in the morning, to remove trapped animals as soon as possible. Mammal carcasses are prone to rapid decomposition in high temperatures, and they may be susceptible to ant damage or maggot infestation if left in traps for an extended period before retrieval. Pitfall traps are particularly suitable for collecting shrews (Abe 1992b; Koyasu 1998);

however, if this type of trap is used as a kill trap, it should contain a sufficient amount of water to ensure that the captured animals do not suffer for a long period before death (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987).

Small "body-gripping" traps were historically used as kill traps for chipmunks and tree squirrels (Abe 1991a). However, body-gripping traps and comparable foothold traps are now cited as examples of non-humane models that do not kill animals quickly. Therefore, their use as hunting tools is prohibited by law. Although their use is exceptionally permitted for scientific capture, they should be avoided because of the possibility of damaging specimens. Alternative, more humane traps should be selected where available.

For mammals such as rabbits, hares, squirrels, small to medium-sized carnivores (Nagorsen and Peterson 1980), and large mammals such as deer (AVMA 2013; Suzuki et al. 2018), shooting may be more humane than trapping (Nagorsen and Peterson 1980). To use guns for scientific collection, researchers must first obtain a hunting license, specifically a first-class gun hunting license for use of firearms or a second-class gun hunting license for air guns, as set forth in Article 39 of the WPML. Moreover, researchers must abide by the regulations on the possession and use of guns, known as the Act for Controlling the Possession of Firearms or Swords and Other Such Weapons and 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, a law entitled the Explosives Control Act (*Kayaku-rui Torishimari-hou*) specifically regulates the possession and storage of ammunition. Researchers must comply with these laws and gain sufficient experience to use guns properly and safely.

In the scientific collection of large cetaceans, Japan employed whaling guns loaded with explosive harpoons during its research whaling activities from 1986 to 2019. The International Whaling Commission recognizes a harpoon gun charged with an explosive called penthrite (i.e., pentaerythritol tetranitrate) as one of the most humane killing techniques for whales (International Whaling Commission 1981). If an explosive harpoon does not instantly kill the whale, a second harpoon or a 9-mm (0.35-in) or larger caliber rifle may be required. Cetacean species are not subject to the WPML; therefore, regardless of the method used to kill 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. Notably, research whaling is no longer being performed following Japan's withdrawal from the International Whaling Commission; thus, killing whales for research purposes is virtually impossible.

3-2. Collection Using Live Traps and Nets

Live trapping is often a preferred method of sampling mammals in certain fields of research such as karyotyping, biochemical and genetic studies, ectoparasite studies, and mark–release–recapture ecological studies. One merit of using live traps is the ability to capture only the target species and number of specimens needed for the study, while all other unwanted animals are released to the wild unharmed. However, it is important to select the correct type of live trap that works most efficiently for its intended catch. For example, the trapping rates of *Crocidura* shrews are significantly higher than those of *Sorex* shrews when Sherman-type live traps are used.

Live traps are usually box- or tube-shaped containers made of various materials such as aluminum, zinc, or wood plates, wire mesh, and/or plastic. They are designed to lure an animal into the trap container, causing the animal to contact a trigger that closes the door of the trap to contain the animal. Commercially available brands include Sherman, Havahart, Longworth, Penlon, and Tomahawk. Live traps can be ordered directly from these manufacturers, through domestic retailers (see Murakami 1992), or by international mail order (e.g., Carolina Biological Supply Company; https://www.carolina.com). Products similar in shape and function to foreign brands are also manufactured and sold in Japan (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 Iseki 1997), whereas wire mesh traps are usually used for the live capture of larger mammals such as nutrias (Miura 1992) and wild cats (Izawa 1990).

Simple techniques for collecting moles include live capture by ambush or with pitfall traps. However, tube-shaped live traps, such Nishi traps and Konishi traps, are more reliable and often used by mole researchers (Abe 1992b; Yokohata 1998; Kawaguchi 2004). Overviews of live trapping techniques for Soricidae species have been provided by Abe (1992b), Koyasu (1998), and Motokawa (1998), whereas those for carnivores have been summarized by Kaneko and Kishimoto (2004). Wooden box traps,

as opposed to metal traps, are preferably used for mustelids because they prevent the trapped animals from damaging their teeth in their attempt to break the trap open (Sasaki 1990). For the live capture of large mammals using foothold traps, the jaws of the trap must be modified by cushioning or padding with rubber for softer contact (Kuehn et al. 1986; Nakazono and Doi 1989; Ikeda 1989; Kaneko and Kishimoto 2004), and the traps should be frequently checked (at least twice daily) (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987). Giant flying squirrels can be caught using a modified sweep net (Baba 1988). Box traps are often used to catch live hares; a method for chasing them into a stretched net has also been described (Yamada et al. 1988).

It is important to select a live trap that is sufficiently large to allow the target species to move within the trap without suffering. To prevent the animal from becoming exhausted due to decreased body temperature during winter, a sufficient supply of food and nesting material must be placed inside the trap, and the whole trap should be covered with heat insulation material. Each live trap should be checked regularly at an adequate frequency to minimize accidental death or exhaustion of the trapped animal. Live traps for nocturnal species should be set before dusk and checked as soon as possible after dawn. They should then be closed during the day to prevent accidental capture of diurnal species (Animal Care and Use Committee 1998). Live traps for diurnal species should be shaded to avoid full sun exposure and should be checked frequently (Ad hoc Committee on Acceptable Field Methods in Mammalogy 1987; Koganezawa 1989).

During routine walks, close attention should be focused on checking traps to avoid overlooking any live animals caught in the traps. At the end of the fieldwork period, all traps must be retrieved, and temporary field markers such as colored tape should be 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 initial setup, regular checking, and final retrieval. This practice will reduce the risk of overlooking any captured animals during regular trap inspection or leaving behind a trap at the end of a trapping session.

Highly frequent inspections are required when capturing live small mammals using pitfall traps (e.g., about once hourly for shrews). Bait should be placed in a pitfall trap to prevent starvation. Additionally, because the mortality rate of captured animals is

higher when the animals become wet by rain, the top of a pitfall trap must be covered to provide shelter (Pucek 1981).

Various types of live traps are conventionally used for large mammals. 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 effective. These animals can also be driven into an enclosure using a corral trap system in conjunction with a metal fence structure (Ito et al. 1989; Nakatani 1989; Kaji et al. 1991). For deer, a modified corral trap system can be used to enclose the animals within cloth curtains that rise when the animals enter the enclosure (Uno et al. 1996). Snare traps are conventionally used for wild boar (Nakatani 1989). A disadvantage of using metal cages as bear traps is that the captured bear is likely to damage its teeth by biting the metal bars of the cage (Yoneda et al. 1996). By contrast, a bear is unlikely to be injured when captured in a barrel trap (Mano et al. 1990). Similarly, when a metal fence corral trap is used to catch deer, there is a risk of the animal crashing into the metal fence, leading to major injury or death. A 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 for the capture of live wild boars because of high injury and mortality rates (Nakatani 1989). When live-catching large mammals in box traps or drum cages, the trap-checking frequency can be reduced by attaching a transmitter to the drop door or monitoring the trap with a network camera. A remote control system has also been devised for closing the entrance of the trap (Nakamura et al. 2019).

For the live capture of large wild animals, all necessary procedures must be performed within the shortest time possible after capture or the animals may become violent and be injured. The researcher must exercise vigilance when capturing and immobilizing animals to prevent capture myopathy. It is essential to have a rapid-acting treatment ready for any symptoms of capture myopathy that may necessitate intervention (Suzuki 1999).

Mist nets, harp traps, insect nets, and direct catching by hand are mainly used for live capture of bats. As with other mammals, a collection permit is required to capture bats for scientific research purposes. In addition, permission must be obtained from the

Minister of the Environment to use a mist net regardless of whether the bat is a rare or common species (cf. WPML Article 9, Paragraph 1, Item 3). This collection permit is also required for the purchase of the net (WPML Article 16, Paragraph 2, Item 1). The Bat Study and Conservation Group of Japan (1998) provides useful information on how to purchase mist nets, as well as techniques for using it to catch bats. Mitchell-Jones and McLeish (2004) also provide helpful information on techniques for using a mist net. The recently popular methods of using harp traps, insect nets, and direct catching by hand require permission from the Minister of the Environment for endangered species and from the prefectural governor for common species.

It is important to adopt the most appropriate capture method for each bat species according to its specific habitat (Mohri 1988; Kunz and Kurta 1988; Kunz and Parsons 2009). Mist nets and harp traps are mainly used to catch flying individuals outside roosts, whereas insect nets and direct catching by hand are often used inside roosts such as caves. Researchers should generally avoid exploring a cave in which a bat maternity colony is roosting during the parturition and nurturing periods to avoid disturbing 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. Even outside of these seasons, bat collection within the roost should be minimized because it may lead bats to abandon the roost.

To force bats out of residential structures, a special device known as a “one-way door” may be used (Gelfand 1997; Tuttle et al. 2020), or the entrance to the structure can be blocked after all bats have left the nest (Yasui 2020). However, these methods should not be applied during the parturition and nursing periods because they may lead to higher mortality of flightless pups, particularly if applied in the summer breeding and nursing months of June, July, and August. A collection permit is not required to build a bat house (a shed or box designed to shelter a bat roost) if the house is meant only for the conservation and observation of bats and the project does not involve direct handling of bats. However, permission from the landowner and property manager must be obtained before building a bat house on private property. In addition, a collection permit is required if fieldwork entails temporary or permanent capture of bats from a bat house to gather data. It is important to select the most appropriate type of bat house with respect to its materials and size and to select a suitable location to install the bat house. Additional

devices that will protect the bats from attacks by potential predators should be placed in the study area (Gelfand, 1997; Tuttle et al. 2020). Overall, bat research requires familiarity with the research methodology peculiar to this group of mammals, including trapping techniques; therefore, investigators should be well versed in the ecological and behavioral study techniques (cf. Kunz and Parsons 2009) for the specific bats of interest.

3-3. Post-Capture Euthanasia

When euthanizing a mammal captured in the wild, the most ethical and humane technique possible must be used (as a legal justification behind this statement, nonbinding rules regarding animal euthanasia are stipulated in Articles 40 and 41 of the revised Act on Welfare and Management of Animals; see Animal Welfare and Management Laws Study Group 2001). Euthanasia is generally defined as 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 [No. 105 of the Ministry of the Environment notification (https://www.env.go.jp/content/900479596.pdf); see also Asano et al. 2006]. Various agents and methods of euthanasia are available, such as administering an overdose of an anesthetic, 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). The selected technique should be the most humane method that, if at all possible, would not subject the animal to pain or suffering while still meeting the study purpose. Historically, the administration of ether was one of the most frequently adopted procedures, but the use of ether is not permitted today because of its toxicity to both humans and animals as well as its irritative, explosive, and flammable attributes. Sevoflurane and isoflurane, which are less stressful to mammals, should be used as alternatives (Marquardt et al. 2018). Pentobarbital is another anesthetic commonly used for euthanasia.

A high concentration of carbon dioxide is often used as a method of euthanasia, but it is usually limited to laboratory use. A sprayable carbon dioxide gas manufactured Nitto Kagaku can be carried outdoors and is convenient for field surveys. Notably, carbon dioxide gas can cause pain in some species because it combines with water in the nasal mucosa to produce carbonic acid. Additionally, burrowing and diving mammals such as rabbits have been shown to be tolerant to carbon dioxide exposure (Suzuki and Kurosawa 2005). Therefore, an appropriately high concentration and time must be used to prevent revival of the animal.

Euthanasia must be performed outside the perceptive range of other captive animals to avoid triggering fear or causing undue stress (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). Researchers should direct any questions regarding euthanasia techniques for particular study animals to 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 was provided by Kagiyama (2008). It is advisable to adhere to any guidelines established by the institution to which the researcher belongs because specific protocols may be enforced. Furthermore, the American Veterinary Medical Association revised its Guidelines for the Euthanasia of Animals in 2020 (AVMA, 2020). This is an informative reference that covers small to large laboratory animals, livestock, and wild animals.

Thoracic (cardiopulmonary, cardiac) compression is a relatively common method of euthanasia that is used when small mammal specimens must be prepared from live individuals collected in the field. The axilla is grasped from behind with the thumb and index/middle fingers of one hand, and the other fingers are used to support the body. The body is further wrapped by the four fingers of the other hand, and the thumb is placed on the ventral border of the sternum. All fingers are then tightened and held until the heart and lungs stop functioning. Loss of consciousness is rapid, leading to death. This method is approved by the guidelines of the American Society of Mammalogists (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016) and is not considered to cause long-term anxiety or pain. Moreover, it does not affect subsequent measurements or damage the specimen. However, it should be performed with the proper technique only after receiving guidance from an experienced person. Its

use in laboratories, where alternative euthanasia methods are available, is unacceptable.

Although cervical dislocation is another method sometimes used in the field, it is not recommended for the purpose of preparing specimens because there is a high possibility of significantly altering subsequent measurements and breaking the skull or skin.

4. Specimen Data

Field data associated with mammal specimens that have been collected, examined, and preserved for systematic studies and other research purposes should be properly stored. For example, field data from specimens that were originally captured for field surveillance can further provide a wide variety of biological information, such as geographic variations in morphology, age variations, age determination, age structure within a population, estimation of the breeding period based on age determination, reproductive condition, litter size, diet and feeding habits, nutritional status, quality of a population, and parasites (Abe 1991b). Moreover, in closely related study areas such as morphology, genetics, biochemistry, and parasitology, the preservation of study materials as voucher specimens not only ensures the taxonomic underpinning of a study but also enables correspondences with future taxonomic revisions.

Therefore, regardless of the main study purpose of collecting mammal specimens, the documentation of basic field data for each specimen (e.g., collector's serial number, species identification, sex, measurements, locality, date collected, method of collection, and other remarks) should be performed as a part of the daily fieldwork tasks. These primary records should be archived together with physical specimens. Some of these specimens should be collected as samples for genetic analysis and stored in a public institution such as a museum along with the date and location of capture, and information on the individual, to enable verification at a later date. However, uncertain data should never be recorded based solely on speculation, even if the investigator is busy handling data from multiple specimens. For example, if the sex of a specimen cannot be determined in the field, it should be indicated with a question mark in a field notebook or field catalog (Nagorsen and Peterson 1980).

4-1. Recording Data

Data associated with field collection activities is usually recorded in a notebook or dedicated data form. Martin et al. (2001) recommended organizing field notes into three separate sections: a journal, a field catalog, and species accounts. The journal is used as a field diary in which all collecting activities and observations are documented on a daily basis. The field catalog is useful for recording specimen data, such as measurements, for each animal collected. These data are recorded in the order of collection serial numbers assigned to each specimen. A species accounts section includes detailed observational records on particular species that are captured (Martin et al. 2001). It is important to archive a journal for all types of fieldwork and to document a field catalog on printed catalog sheets rather than in a blank notebook (British Museum 1968; Nagorsen and Peterson 1980). If custom printed catalog sheets are not used for a field catalog, then commercial A4-sized horizontal-ruled data-recording sheets (e.g., KOKUYO Accounting Pad SHIYO-26N) are recommended for use as catalog data forms given their ease of use and reasonable price. Today, it is acceptable to record field data electronically using spreadsheet software such as Microsoft Excel or database software such as File Maker. Notably, however, electronic data should be backed up and ideally printed out on paper to be filed physically to ensure its long-term preservation.

Three data categories should appear as column headings on each catalog sheet, as follows:

1) Field Number: This is a unique number that is assigned to each specimen and recorded on the catalog sheet in the field. Unless an established specimen numbering system is already being followed, the collector's serial numbers should be used (i.e., a series of sequential numbers independent of the field visit, locality, species, or collection date). The field number should be permanently written on a field tag attached to the specimen using a pencil, India ink, or permanent ink but never a ballpoint pen, felt-tip marker, or fountain pen loaded with a water-based ink cartridge.

2) Catalog Number: This is a number that is assigned and recorded once the specimen has been accessioned to an institutional collection. Thus, catalog numbers are not recorded in the field.

3) Species Name: The species name is used as a record of species identification (Japanese name or scientific name) in the field. Useful references for identification of Japanese mammals include colored illustrations (Imaizumi 1960; Ohdachi et al. 2015), keys (Maeda 1983; Yoshiyuki 1989; Abe et al. 1994, 2005), and skull illustrations (Abe 2000).

Standard formats for recording sex, weights and measurements, and locality are discussed in Sections 4-3, 4-4, and 4-6, respectively. Data on reproductive condition, habitat, and field observations are either documented as three separate categories on a catalog sheet or combined under "Remarks" (see Sections 4-5 and 4-7).

When specimens are to be deposited in a museum collection, the associated documentation should also be permanently archived by the museum, including all catalogs, field notes, photographs, and maps of collecting sites. Because catalog sheets and field notes are often the only sources of information for specimens, they should be well organized, legible, and as accurate as possible (Nagorsen and Peterson 1980).

4-2. Specimen Labels

High-quality paper that is resistant to alcohol and formalin solutions should be used for specimen labels. For example, 200 μ Color Water Resistant Paper manufactured by Shisei Co., Ltd. has excellent durability and is sufficiently thick to be attached to a specimen by string. Additionally, the writing utensil used should be resistant not only to alcohol and formalin but also to water and oils. A pencil is a versatile tool in this respect, but care must be taken because pencil marks are vulnerable to scraping. Considering the deeper penetration of a pen into paper, the use of a pen mounted with carbon ink is recommended. Labels are attached to many different types of preparations: 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 another collector's specimens (Nagorsen and Peterson 1980). Abe (1991b) outlined the data items to be recorded on a specimen label. Each data item should be transcribed as completely as possible from the catalog onto the label. An alternative method is to attach a label containing only the specimen number and preserve the individual information in a notebook or digital file separately; however, this is not recommended

because of the possibility of losing the notebook or file. In addition to the collection number, if at least the collection date and location are recorded on the label, the absolute minimum individual information of the remaining specimen can be rescued. Information such as species name, sex, and measurements can be inferred from the specimen itself, but it is difficult to reconstruct collection dates and locations. Methods for determining which data items to include on a specimen label as well as the proper format and style to be used can be found in the examples provided by Okada (1940), the 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 the specimens 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. In published literature, voucher specimen numbers and identifiers for tissue or parasite samples should be cited to facilitate cross-referencing.

4-3. Sex Determination

It is often possible to determine the sex of a medium-sized to large mammal based on inspection of the external reproductive organs, such as the vaginal opening, scrotum, and penis; this can also be accomplished for small mammals during the breeding season (Imaizumi 1970; Abe 1991b). The external reproductive organs of cetaceans are housed within a genital slit; however, males and females can be differentiated based on the distance between the anus and genital slit, which is relatively longer in males than in females (Nagorsen and Peterson 1980). Similarly, rodents can be sexed externally by the distance between the anus and reproductive organ (clitoris or penis) (Nakata 1986; Abe 1991b). By contrast, juveniles and small mammals in the non-reproductive season have poorly developed external reproductive organs. Therefore, the internal reproductive organs of a specimen should be inspected to reduce the chance of false sex determination (Imaizumi 1970; Nakata 1986; Abe 1991b). To sex a specimen by its internal reproductive organs, the ventral side of the body must be dissected; this is normally performed after the specimen has been skinned in preparation for a skin study. Sex can

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

4-4. Body Weight and External Measurements

Specimens should be weighed as soon as possible before beginning preparation. Small mammals are measured in grams, and larger mammals are measured in kilograms. A scale is used to weigh small animals; a portable digital scale (e.g., Tanita Mini Scale 1476 model) is convenient for animals weighing up to 100 g. Backup batteries should be available whenever a battery-powered scale is used in the field. The body weight of a large mammal is difficult to obtain in the field. Normally, a portable mechanical spring scale is used for this purpose. When a power source is available, an electronic livestock scale is useful. Battery-powered scales that measure up to 100 kg as well as scales that have a waterproof function are available commercially. When only general equipment capable of weighing up to approximately 20 kg is available, each part of the individual animal can be weighed separately when performing dissection. In such cases, it is important to record that the weights are inaccurate because of the loss of blood and other fluids. When recording such measurements, the numbers are conventionally written in parentheses.

In the North American system (as established in Canada and the United States), the standard external measurements for small mammals are total length (TL), length of the tail vertebrae (LTv), hind foot length (HF), and ear length (E) (Corbet and Ovenden 1980; Nagorsen and Peterson 1980). In this system, HF includes the extent of the claw or nail without exceptions [hind foot *cum unguis* (Hfcu)]. By contrast, in Europe (including the United Kingdom), head and body length (HB) is more commonly measured than TL, and HF does not include the extent of the claw or nail (Corbet and

Ovenden 1980; Nagorsen and Peterson 1980; Pucek 1981; Imaizumi 1986). When measuring tail length, definitions of the tail's point of origin vary regionally. 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). In Europe excluding the United Kingdom, tail length is defined as the distance from the center of the anus to the tip of the tail (Pucek 1981). In both systems, any tuft of hair extending beyond the end of the tail is excluded from the measurement.

Japan historically used both the European method and the amended North American method (tail length measured with North American method versus HF measured with European method). In recent years, however, the amended North American system has been more commonly used 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 recently published field guides and textbooks most likely follow the amended North American method in indicating the tail length and HF without the claw [hind foot *sine unguis* (HFsu)] as explained above. However, very few countries other than Japan have adopted the amended North American method as a national standard for measuring mammals; thus, researchers should be aware that it is not regarded as an international standard. Although the British method is similar to the amended North American method in terms of HF because it excludes the claw and tail length and defines the origin as the root of the tail vertebrae, these methods differ in that in the British method, the tail is measured without bending it toward the back (Corbet and Ovenden 1980; Burton 1991). In China, the European method is adopted as the standard for measuring mammals (Wang and Ganyun 1983). It is technically difficult to measure foot length exclusive of the hoof (homologous to the claw) in ungulates, making the North American method the only practical means to measure the HF of these animals. Thus, the North American method is the only system that allows the body measurements of terrestrial mammals, including ungulates, to be documented in a consistent manner. Technical instructions for measuring small mammals in accordance with the amended North American method are provided below (cf. Abe 1991b).

1) 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.

2) 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 the short side of a ruler backward along the lower back of the animal until it stops naturally 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).

3) HB: Subtract the LTv value from the TL value.

4) HFsu: Stretch the hind foot toes and measure the distance from the tip of the heel to the extremity of the longest toe (excluding the claw).

5) 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).

In addition to these basic measurements, forefoot length and forefoot width are commonly measured in soricomorphs. Forefoot length is measured from the rear end of the forefoot sole to the tip of the longest finger, excluding the claw. Forefoot width is defined as the longest width across the forefoot sole. For chiropterans, the forearm length, tibia length, tragus length, and wingspan are usually also measured. Forearm length is the distance between the wrist joint and the elbow joint. Tibia length is measured from the knee joint to the distal end of the tibia. Tragus length normally refers to the distance from the base to the tip of the tragus along its inner edge. Notably, Handley (1988) defined two measurements for the tragus: the total length of the tragus and the length of the tragus blade. The former is defined as the tragus length along its outer edge, whereas the latter is defined as the tragus length along its inner edge. The wingspan is the greatest width across the wings when the bat is positioned with both wings fully spread out. For large animals, the height at the shoulder, chest girth, and

other parts are measured. Height at the shoulder is measured straight downward from the upper edge of the shoulder blade to the bottom of the feet while the animal stands on all four feet. The external measurements of cetaceans in Japan can be downloaded from the website of the National Museum of Nature and Science (https://www.kahaku.go.jp/research/db/zoology/marmam/recording_sheet/). The site provides a detailed record sheet for each family of cetaceans inhabiting Japanese waters. Hamada (1986) provided standard external measurements of the Japanese macaque. Most 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). Therefore, researchers should consult these diagrams and instructions during measurements until they are familiar with the specific methods used to measure each part of the body and the corresponding set of metric data to be collected.

Importantly, variations in the morphometric values recorded by different investigators can lead to significant biases in the calculation of parameters from external and skeletal measurements. Interobserver measurement error can be particularly problematic for body parts bearing significant individual variation or those for which the origin and/or endpoint of a particular metric are indistinct and difficult to define (Palmeirim 1998; Blackwell et al. 2006). Therefore, when performing external measurements, the researcher should measure the same body part at least twice and use the mean value for analysis (Blackwell et al. 2006).

4-5. Reproductive Condition Data

Observation of the internal reproductive organs provides important biological data regarding an animal's reproductive condition. Such data provide an understanding of the 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 at reproductive maturity (Nagorsen and Peterson 1980).

Checking the size of the testes and visually inspecting the epididymal duct are simple methods for determining the reproductive condition of males. For a fresh specimen that was recently euthanized, the longest and shortest diameters of the testes

should be measured after opening the abdominal cavity. When it is difficult to accurately measure the dimensions of the testes, including those preserved in fluid, the researcher should at least make a note regarding the condition of the testes with respect to whether they have descended to fill the scrotum; i.e., scrotal (+) or non-scrotal/abdominal (–). For small mammals, the reproductive condition of males can be determined by inspection of the epididymal duct. If the duct is visible (a positive indicator of sperm production and storage), the individual is considered reproductively active (+). If the duct is not visible, mature sperms are not accumulating sufficiently in the testes, and the animal is considered reproductively inactive (–). To determine the reproductive condition of a male with a high degree of certainty, the researcher should prepare slides with a sperm smear or testis tissue for microscopic observation. Regardless of the method adopted, the reproductive condition of the animal should be observed and documented in a field catalog or notebook immediately after its capture.

With respect to the reproductive condition of females, a pregnant or lactating animal is clearly considered reproductively active (+). Another reliable criterion is whether the vaginal orifice is open (+) or closed (–). The condition of the uteri should also be examined. Other reproductive criteria include whether fetuses or placental scars are present (+) or absent (–). If present, the size and weight of the fetuses and the number of placental scars on the right and left uteri should be recorded. Moreover, the nipples and luteal state of the ovary should be externally observed and recorded as either developed (+) or undeveloped (–).

Methods of recording the reproductive condition of mammals as described 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 the expanding field of biodiversity research, including traditional studies emphasizing geographic and population variation. When recording localities in Japan, the researcher must document at least the prefecture, city, town or village, and geographical features such as lakes or mountains. The exact geographic coordinates of the collection point should be recorded in latitude and

longitude, to at least the minute. Latitude and longitude can be obtained with reference to the standard base maps provided by the Geospatial Information Authority of Japan (formerly the Geographical Survey Institute) at a scale of 1:50,000 or 1:25,000. Alternatively, the use of a car navigation system or device equipped with a geographic positioning system, if available in the field, enables a researcher to read the latitude and longitude of a collection site in real time. The online map browsing service provided by the Geospatial Information Authority of Japan may also be useful (https://maps.gsi.go.jp/). Additionally, it is advisable to reference a collection locality using the assigned third mesh code number, which corresponds to each 1 km × 1 km land grid on a map of Japan developed for the National Survey on the Natural Environment (Environmental Agency of Japan, 1997). For countries outside of Japan, it is preferable to document the place name as an administrative unit along with its latitude and longitude in accordance with the standard recording system described above. Ideally, mapping the points of collection on a relatively large-scale contour map (1:50,000 to 1:200,000) attached to a field catalog or notebook is recommended.

4-7. Recording Additional Data

The habitat features at collection localities should be recorded because such data provide an indication of the ecological distribution of a particular species. These data include the landscape, dominant vegetation, elevation, soil condition, and other related information. The method of collection (e.g., trapping, netting, or salvage of carcasses) should also be recorded, but this is not necessarily required for each individual specimen when all the specimens were obtained using the same method in a single field session. In such cases, it is sufficient to summarize the specimen collection method at the top of the first entry of a session in a field catalog or notebook; the same information does not need to be repeated for the remaining data entries. Other behavioral and ecological observations can also be recorded as supplementary data in a field catalog or notebook. Specific examples include the time of specimen capture, climatic conditions at the time of collection, vocal communication, and any field signs observed at the collection site. Making notes of miscellaneous information may seem cumbersome, but such data often become an invaluable reference for natural history studies in subsequent years.

Recording large amounts of detailed information about specimens may lead to

unexpected discoveries when the specimens are later re-examined in a laboratory or museum. For example, unusual coat colors (such as balding or whitening of the tail tip) or bodily damage (such as hair loss or chafing) should be recorded to ensure that these abnormalities are not due to sampling or transportation. With respect to development, it is possible to determine whether a specimen is a subadult or an adult based on the wear condition of its teeth. Young bats can be identified by observing the epiphyseal closure of the joint between the metacarpal and proximal phalanx through the wing. However, because ossification is completed within a few months after birth (depending on the species), the judgment period is relatively short.

Photographs of a collection site and the animals obtained are particularly valuable when researching extremely rare species in the wild or an individual animal showing unique pelage variations. Close-up photography is an effective means of documenting the facial region of a live-caught mammal, as such details may shrink or lose 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 for preservation in a collection room are classified into three different categories according to the preparation technique: the skin and skull (which may include a partial post-cranial skeleton), the complete skeleton including the skull, and fluid-preserved whole body or partial organ specimens. Each preservation technique has strengths and limitations and should be chosen based on the particular research purpose.

5-1. Preparation of Fluid-Preserved Specimens

A whole animal carcass is pickled and preserved in 70% ethyl alcohol or 10% formalin solution (i.e., 4% formaldehyde aquatic solution) within an airtight container. Body measurements of the specimen must be obtained before it is placed in the fixative agent because the chemical process of fixation causes hardening and shrinkage of the body. A partial ventral incision should be made in each specimen to facilitate infiltration of the fixative into the body cavity (Abe 1991b). Formalin solution is stronger than alcohol for specimen fixation; however, formalin breaks down into formic acid over

time (within 1–3 years), leading to decalcification of the teeth and bone. To prevent this degradation, specimens must 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 the solution is difficult to switch, it can be neutralized by adding ammonia solution, marble fragments, or hexamethylenetetramine (Hayashi 1981). Notably, Bouin solution requires special caution because its main chemical component, picric acid, is explosive.

Direct fixation and preservation of specimens in ethyl alcohol solution instead of initial fixation in formalin makes it possible to subsequently obtain a tissue sample for DNA analysis. Although the direct preservation of specimens in alcohol of exactly or nearly 100% concentration is considered optimal for preparing DNA samples, this technique may lead to shrinkage of the musculature, causing skeletal deformation. Therefore, it is advisable to separately preserve specimens for DNA analysis, such as minced liver tissue, when fluid-preserved specimens are prepared, using an appropriate solution, such as 70% ethanol or by initial fixation with 10% formalin that is then replaced with 70% ethanol after washing with water. According to Simmons (2014) and Timm et al. (2021), high-concentration ethanol (≥ 96%) is unsuitable because it degrades DNA. For this reason, azeotroping with benzene was historically practiced to achieve high alcohol concentrations, a process that can denature DNA if the solution is not properly handled (Dr. Nobuaki Nagata pers. comm.). Although the use of benzene is currently prohibited in Japan, high-concentration ethanol is commonly used for DNA preservation. Notably, because ethanol may still be synthesized using benzene azeotropy overseas, caution is warranted when obtaining highly concentrated ethanol outside of Japan.

Specimens are most effectively stored in a large, colorless, transparent glass container, such as a wide-mouth container with an airtight seal similar to those commonly used for preserving plum wine. Alternatively, a smaller glass container with a cap lined with an inner closure, commonly known as a “mayonnaise jar,” can also be utilized; these are typically available through scientific laboratory equipment and supply vendors. Recycled food containers, such as emptied powder coffee bottles, are inadequate and should never be used to store specimens because of their non-airtight sealing (which causes evaporation of fixative solution) and sometimes metal lids (which are prone to rusting). Labels associated with fluid-preserved specimens should always

be soaked in solution together with the specimens. Motomura (2009) discussed in detail various techniques for the preparation, management, and photography of fluid-preserved fish specimens. This information is also generally applicable to fluid-preserved mammal specimens.

5-2. Preparation of Skins

Standard techniques for the preparation of museum skins are discussed in the literature (e.g., Abe 1991b). Medium-sized to large mammal specimens are often preserved in a dry state, as flat hides or tanned skins, for scientific use. Three different types of preservation methods are used for small mammals:

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, dried alum, or a mixture of equal parts camphor and either borax or alum). The skin is then stretched flat on a board, pinned, and left to dry.

2) Study skins: The skin is incised longitudinally along the posterior abdominal midline, but not all the way anteriorly. It is then peeled off the body and cleaned by removing fat and muscle from its interior. After the application of a dry preservative (such as those described above) to the inner side, the skin is stuffed with cotton to achieve the appropriate level of hardness. The stuffed skin is then sewn with 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 feet. The tail vertebrae and all four legs are often replaced with wire, bamboo rods, or gramineous grass rachises to maintain the skin's 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 then cut off without damaging the skin. Finally, the whole skin is removed from the body. The interior aspect of the skin is treated with a dry preservative as mentioned above. The skin is stretched over 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

stainless steel thread or staples. The size of the cardboard is based on the body size of the animal. The cardboard also provides a surface for specimen labeling and writing field data and measurements.

Various manuals provide detailed step-by-step techniques and diagrams for preparing mammal skins (British Museum 1968; Imaizumi 1970; Hashimoto 1979; Nagorsen and Peterson 1980; Pucek 1981; Honda 1985; Abe 1991b; Martin et al. 2001). Researchers should refer to these references often until they are accustomed to preparing skins.

5-3. Preparation of Skulls and Skeletons

Once the skin has been prepared using one of the methods described in Section 5-2, the body can be stored with a label in 70% ethanol, which is convenient for whole-skeleton preparation). Alternatively, the head can be cut off of the skinned body at the atlas joint and dried for transportation. Care should be taken to avoid breaking the occipital region of the skull during the procedure. For relatively small mammals with fragile skulls that can be easily damaged (e.g., soricomorphs, murids, and bats), the skull should be dried without removing any flesh. For animals larger than a squirrel, the brain, eyeballs, tongue, and thicker layers of muscle should be removed from the head before drying. Heads that are separated and dried in this manner during fieldwork should be immediately labeled with the same number as that recorded on the specimen label attached to the matching skin before they are transported to the laboratory along with other specimens upon completing fieldwork. If these dry heads are not immediately ready to be cleaned, they can be kept dry in insect-, dust-, and light-proof containers.

One of the easiest and most effective means of cleaning a skull is to boil it in hot water to sufficiently loosen the attached muscle and soft tissue. The softened tissue can then be removed by hand with a pair of forceps, and the 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 be used to effectively to clean the skull. It is preferable to use a relatively large beetle species, such as *Dermestes maculatus*, which can be easily managed to prevent leakage, rather than a smaller species such as *Anthrenus verbasci*, a well-known pest for museum

specimens. When cleaning skulls of medium-sized and large mammals, the following procedure is recommended to prepare high-quality specimens: pretreat the head by boiling it in hot water to remove most of the flesh, soak the skull in an aquatic solution of proteinase overnight to further break down the soft tissue, and finally wash the skull to completely clean it of soft tissue (Hachiya and Ohtaishi 1994). The proteolytic enzyme Tasinase N-11-100, originally recommended by Hachiya and Ohtaishi (1994), is no longer available. As a substitute, Bioprase AL-15-FG (manufactured by Nagase ChemteX) can be used at an optimum temperature of approximately 50°C, which is achieved using a water heater with a thermostat. For large mammals, aerobic bacteria can be used instead of an enzyme. A maceration bath is created by heating water to 37°C–38°C with constant aeration achieved by pumping small air bubbles into the water. After the skull has been soaked under these conditions for a few days, it is completely cleaned of soft tissue by the bacteria proliferating within the system. The use of an enzyme or aerobic bacteria requires adequate ventilation because it generates a strong odor during processing. Caustic chemicals, including bleach, pipe cleaning products, and strong alkaline agents (e.g., sodium hydroxide or potassium hydroxide), should not be readily used to remove flesh from a skull because they can destroy or deform bone and delaminate tooth enamel.

Regardless of the cleaning method applied, the defleshed skull may be bleached for aesthetic purposes. Small mammal skulls should be soaked overnight and medium-sized to large mammal skulls should be soaked for 3–5 days in approximately 10% hydrogen peroxide solution. The skulls should then be thoroughly washed with water and allowed 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 has been cleaned with aerobic bacteria.

Preparation of the skeleton is conducted as follows. The specimen is skinned using one of the methods described in Section 5-2, and the abdomen is cut open to determine the animal's reproductive condition (see Section 4-5). All internal organs are then removed from the abdominal and thoracic cavities because remnant viscera can cause decomposition. Depending on the purpose of the study, various samples are taken from the tissues and organs at this stage. Large muscles are then removed from the remaining body, and the skeletal material is left to dry. The method used to prepare a

skeletal specimen from a dry body is basically the same as that used to prepare a skull, although processing becomes more difficult as the animal's body mass increases. Hachiya and Ohtaishi (1994) provide information on degreasing in the preparation of 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 articulations, sutures, minute bones, and cartilage must be preserved intact, then chemical treatments such as bleaching and degreasing should be minimized.

6. Transportation and Management of Mammal Specimens

6-1. Transportation of Specimens and Data

The transportation methods described below are mainly those recommended by Nagorsen and Peterson (1980) and are based on the assumption that specimens and their data will be transported from a field collection site to a museum facility. Situations differ significantly from one case to another (e.g., when specimens are carried by collectors themselves versus being shipped by a third party such as a mailing service). There are also considerable differences between domestic and overseas transport. Nonetheless, the following basic points apply to most transportation modes. The methods recommended herein are also applicable when completed specimens are moved between museums, among individual researchers, and between museums and researchers.

6-1-1. Fluid-preserved specimens

Wet specimens that have been properly fixed in formalin will maintain their fixed state even when immersed in water for approximately 1 week after washing. Therefore, these specimens can be transported in a moist condition by rinsing them with water for approximately 1 day and night, followed by adding a small amount of water to the container. For alcohol-fixed specimens, considering the flammability of 70% ethanol, it is advisable to include a small amount of low-concentration alcohol and transport the specimens in a moist state. When packing wet specimens, it is essential to include cotton, cheesecloth, or newspaper in each container to fill gaps, preventing the specimens from shaking while preserving moisture within the container.

Cheesecloth is a superior filling material because it can cover the entire surface of

a specimen and effectively keep it moistened. A wide-mouth plastic jar is an ideal packing container for specimen transport. A tightly sealed plastic bag is an acceptable substitute. However, a glass container is inappropriate for specimen transport. If specimens must be transported in a glass jar, the whole container must be packaged in a sturdy box together with enough cushioning material to fill any gaps and prevent the glass from breaking. If plastic bags are used for packing, the specimens should be placed in an inner bag with cotton and fixative solution; this inner bag should be tightly sealed after removing all air. Next, the inner bag should be sealed within another plastic bag, and the double-bagged content is then placed in a metal box (such as an emptied commercial cake box) containing a matching lid firmly secured in place with adhesive tape. If a large gap is present between the metal container and the bagged content, the gap should be filled with cushioning and absorbent materials such as cotton, cheesecloth, and newspaper. The whole package wrapped in this manner is usually compact, and if handled with care to prevent leakage of the fixative solution, it can be shipped domestically by regular parcel post or other delivery services. 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 problematic upon quarantine in some areas; thus, only synthetic cotton should be used. Additionally, the chemical class and quantity of solution contained in air parcels may be subject to strict regulations. When a specimen is received, it must be immediately transferred to an airtight container filled with the liquid in which it was fixed.

6-1-2. Skins and skulls

Ideally, skins should be transported in a container made of sturdy plywood; however, a corrugated cardboard box is also acceptable for specimen shipping purposes. First, the bottom of the container is lined with a sheet of cotton or similar cushioning material to a depth of approximately 5 cm. Several dry study skins are then evenly spread on top of this cotton layer. Another flat layer of cotton is placed on top of the specimens, and additional skins are spread on the cotton. This layering is repeated until the uppermost layer of cotton sits at a depth of approximately 5 cm from the top of the container. If transportation of skins is expected to take more than several days, it may be

necessary to treat the specimens by adding a paradichlorobenzene-based insecticide, commercially known as parazole, in the same shipping container to repel moths and skin beetles and prevent their eggs from being laid on the skins. Alternatively, the specimens can be pretreated to eradicate pests by other non-chemical means such as subjecting them to cold temperatures before packaging them for transport.

6-1-3. Fluid body for skeletal specimens

When transporting the dried head and body of skeletal specimens, the skinned body is fixed with 70% ethanol and transported in the same manner as for fluid-preserved specimens (see above). When preparing skeletal specimens using dermestid beetles after transport, the fluid body should be thoroughly washed with water and then dried. If the skinned body is completely dry and pest-free, with no risk of infestation by insects or their eggs, it can be shipped in the same container with dry skins. Insect repellent should not be applied if dermestid beetles will subsequently be used to prepare the skeletal specimens.

6-1-4. Data

Original copies of field catalogs 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. If such documents are mailed overseas from a country with an unreliable mailing system, the items should be shipped by registered mail.

6-2. Regulations on Importation and Exportation

The Washington Convention, officially known as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), was implemented in 1975 and has been in effect for trade in Japan since November 1980. According to this treaty, both the import and export of endangered and threatened species requiring special protection are prohibited without proper legal permits. On October 1, 2019, Japan adopted the CITES Specified Scientific Facility Comprehensive Approval System, eliminating the need for permission for imports and exports between specified scientific facilities. This regulation applies to all international transactions. As long as a species is

listed as threatened, not only the whole individual body (live or dead) but also the parts thereof (including the skull, skeleton, tissues, cells, and biochemical samples) and their artifacts (including taxidermy) are subject to restrictions. As long as either of the countries involved in the international trade is a CITES party, all wildlife trade undergoes rigorous monitoring to ensure the acquisition of necessary permits. Species subject to CITES regulations in international trade are listed in Appendices I–III. Species listed in Appendix I are considered the most endangered. Those in Appendix II are not necessarily threatened with extinction currently, but their trade requires close monitoring to prevent potential endangerment. Species listed in Appendix III are those recognized by at least one CITES party as requiring trade regulation within their respective jurisdictions. Permits are required from both the importing and exporting countries for international trade of the species listed in Appendix I. All species listed in Appendix I, along with domestic species designated as national endangered species of wild fauna and flora (including 15 mammal species), are subject to domestic transport regulation and require mandatory applications and permits. These 15 mammal species are the Daito flying fox (*Pteropus dasymallus daitoensis*), Erabu flying fox (*Pteropus dasymallus dasymallus*), Bonin flying fox (*Pteropus pselaphon*), little Japanese horseshoe bat (*Rhinolophus cornutus orii*), Okinawan least horseshoe bat (*Rhinolophus pumilus*), eastern bent-winged bat (*Miniopterus fuscus*), Ryukyu tube-nosed bat (*Murina ryukyuana*), Yanbaru whiskered bat (*Myotis yanbarensis*), Tsushima leopard cat (*Prionailurus bengalensis euptilurus*), Iriomote leopard cat (*Prionailurus bengalensis iriomotensis*), Ryukyu long-furred rat (*Diplothrix legata*), Okinawa spiny rat (*Tokudaia muenninki*), Amami spiny rat (*Tokudaia osimensis*), Tokunoshima spiny rat (*Tokudaia tokunoshimensis*), and Amami rabbit (*Pentalagus furnessi*). Legal procedures are handled on a case-by-case basis (e.g., a transaction between museums versus between a private party and a university). It is necessary to consult the Wildlife Division of the Nature Conservation Bureau of the Ministry of the Environment before transporting these species. Further details are provided by the Environmental Agency Wildlife Conservation Administration Study Group (1995). To ship species listed in Appendix II overseas, an export permit from the exporting country is necessary. For species listed in Appendix III, international trade requires an export permit from the exporting country, but only if that country lists the specific species in Appendix III, along with a certificate of origin. All

parties involved in wildlife trade must be knowledgeable about the regulated actions in Japan related to CITES and the list of species in the appendices. Resources such as the Wildlife Conservation Issues Study Group (1988) provide more detailed information. The CITES Conference of the Parties holds a meeting approximately every 2.5 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 to ensure that the particular species of specimens intended for transport do not infringe on these regulations.

Kaneko (2001) provides wildlife import clearance procedures following the Washington Convention, a list of CITES Management Authorities and Scientific Authorities in Japan, and a list of Management Authorities of the Association of Southeast Asian Nations Parties. If any of the formalities concerning CITES are unclear, the researcher should consult the appropriate Scientific Authorities and Management personnel (e.g., for terrestrial animals, contact the Wildlife Division, Nature Conservation Bureau, Ministry of the Environment and the Trade Licensing Division, Trade and Economic Cooperation Bureau, Ministry of Economy, Trade and Industry) (Kaneko 2001). In addition to CITES, it is crucial to be mindful of domestic regulations outlined in the Wildlife Protection and Management Law, specifically Sections 25, 26, and 27, which govern the export and import of individual mammals and their products (for further details, refer to Wildlife Management Study Group 2001). When conducting fieldwork overseas, it is essential to adhere to the laws and regulations of the local country, as well as any relevant international treaties, in addition to complying with CITES (Ad hoc Committee for Animal Care Guidelines 1985).

In October 2010, the 10th Conference of the Parties to the Convention on Biological Diversity adopted the Nagoya Protocol on International Rules for Transfer of Genetic Resources and Access and Benefit Sharing (ABS), which became effective on October 12, 2014. Consequently, when obtaining specimens from overseas, it is essential to secure mutually agreed terms (MAT) on ABS with collaborative researchers in the country and obtain prior informed consent (PIC) from the agency responsible for ABS in the government of the country providing the specimen. For researchers importing specimens, the ABS Support Team for Academia (https://idenshigen.jp) at the National Institute of Genetics offers assistance with the required procedures.

6-3. Collection Management

The management of a systematic collection at a museum involves various activities ranging from the accessioning of specimens to the implementation of preservation treatments (Wiley 1981). Examples of published works in the form of field collection records, specimen catalogs, 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 (2004) of the American Society of Mammalogists established "basic curatorial standards for systematic collections of mammals," which are included in an appendix to these guidelines. 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 considered a potential carrier of zoonotic disease. Similar to diseases typically known as endemic, many of these diseases occur locally. Therefore, creating exhaustive lists of possible zoonoses that could be transmitted during the process of collecting and preparing mammal specimens is not realistic (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). Before researchers begin field collecting, they must consider potential diseases that can be contracted from wildlife in the study area and educate themselves about these diseases. If there is a risk of infection with diseases such as typhus or rabies from animals captured during field activities outside of Japan, it is necessary to consult a medical doctor about vaccination options. Caution is also advised within Japan because multiple zoonotic diseases have the potential to infect researchers directly or indirectly through native animals; these include echinococcosis, tsutsugamushi disease (scrub typhus), Lyme disease, hemorrhagic fever with renal

syndrome, Japanese spotted fever, novel coronavirus (SARS-CoV-2), and severe febrile thrombocytopenia syndrome (SFTS) (e.g., Suzuki and Ikeda 1985; Arikawa and Hashimoto 1986; Arikawa 1996; Takahashi 1998; Asano et al. 2006; Watari and Suzuki 2023). Although not zoonotic, some serious diseases such as swine fever are transmitted between wild and domestic animals via the attachment of pathogens to people, small animals, and objects. Researchers who frequently interact with wild animals should exercise caution. Contagious diseases, particularly emerging infectious diseases, have been perceived as a threat not only to humans and livestock but also to biodiversity in recent years (Daszak et al. 2000). Researchers must act carefully to avoid transmitting pathogens to other people, livestock, and wildlife.

When handling live mammals, researchers must protect their hands with thick cotton or leather gloves and pay close attention to avoid being bitten. Fortunately, the risk of rabies infection from wild animals in Japan is considered minimal. However, outside of Japan, potential rabies vectors include bats, foxes, mongooses, skunks, and raccoons. The 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 capture animals for fieldwork overseas, particularly in areas where the occurrence of rabies has been reported, the animals must be handled with care and presumed to be potentially infected with rabies. Rabies vaccination is highly advisable 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. Each field researcher should be well informed about personal healthcare overseas, including vaccination information, prior to their departure (see Miyazaki 1999). Constantine (1988) describes the health risks and necessary handling measures specifically pertaining to bat research. The International Union for Conservation of Nature (IUCN) issued a guideline to reduce the risk of infection under the suspicion that horseshoe bats are the natural host of SARS-CoV-2, which has spread worldwide since 2020. In addition to postponement of the field survey schedule and the use of alternative survey methods, sterilization of all survey equipment (traps and measurement equipment) is recommended. This guideline is designed more to prevent human-to-bat transmission than to prevent bat-to-human transmission. More information can be found on the IUCN SSC Bat Specialist Group page

(https://www.iucnbsg.org; Japanese translation available on the Bat Study and Conservation Group of Japan website, https://www.bscj.net).

When preparing mammal specimens, common sense precautions can help to reduce the risk of infection. Latex gloves and a disposable paper mask should be worn during dissections. Animal excreta must not be touched with bare hands because it can be a source of infection. Dissection tools should be disinfected using appropriate antiseptic measures after each use. The handling of roadkill also requires precautions against possible infection. If researchers notice the following medical symptoms after specimen collection or preparation, they must consult a medical doctor and report a possible zoonotic infection to obtain an accurate diagnosis: flu-like symptoms, chronic respiratory problems, enlarged lymph nodes, high fever, vomiting, or diarrhea.

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