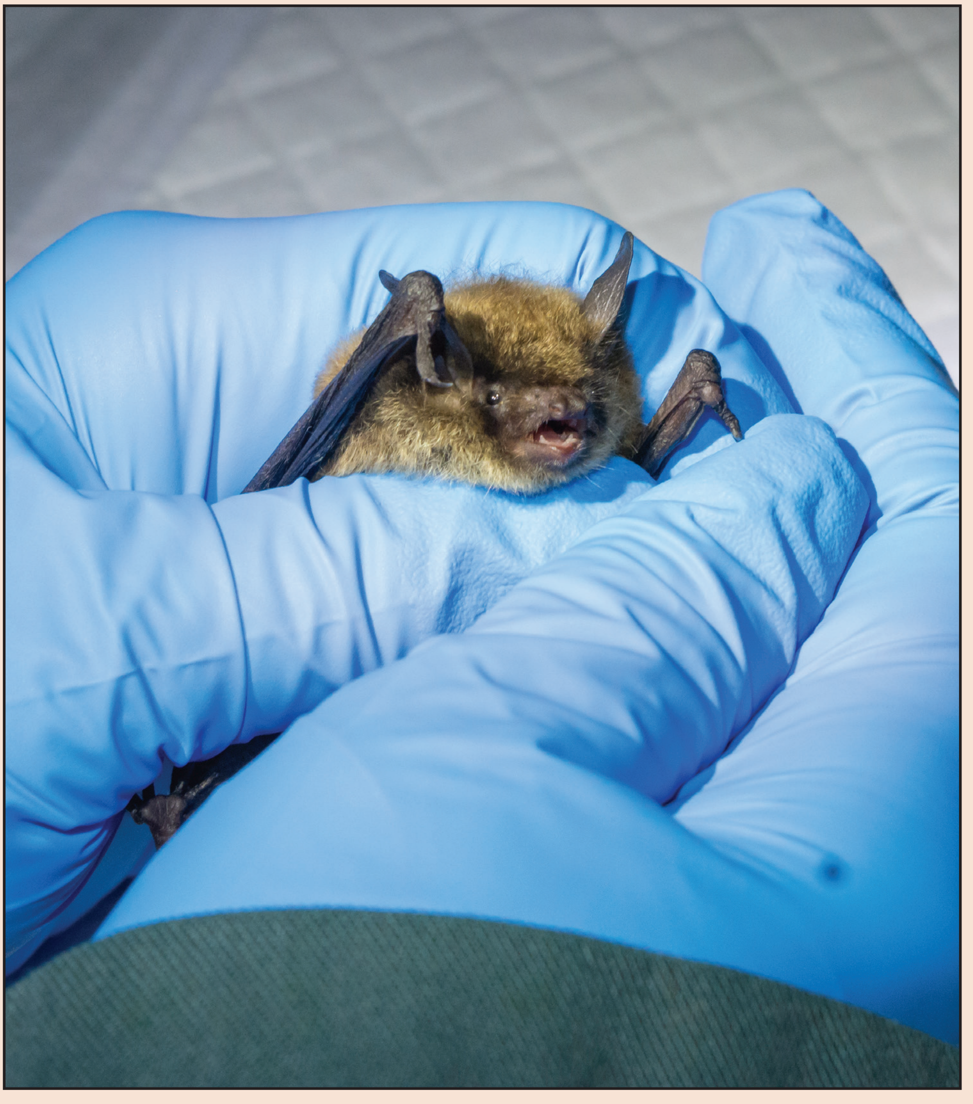
Welfare and Handling Recommendations for Bat Surveys in Canada



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Cover Photograph: Little Brown Myotis in gloved hands, demonstrating safe handling technique. Photograph © Jordi Segers, CWHC.

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Welfare and Handling Recommendations for Bat Surveys in Canada

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Abstract - Concern for bats and their protection has steadily increased globally over the past 2 decades, including in Canada. This heightened interest has resulted in increased population and health monitoring and greater regulatory requirements for bat-related work, compared to the past. There is also increased awareness about bat welfare when handling, particularly with respect to pathogen transmission. Although guidelines for effective techniques to study bats exist, such recommendations rarely mention explicitly how best to prioritize animal welfare. Instead, safe handling practices are implicit, often passed down from mentor to mentee, and the collective wisdom is seldom permanently recorded. Here, we provide recommendations based on consensus reached through review of existing published materials and thoughtful discussion among leading experts with cultural knowledge that spans decades. These recommendations are not meant to be prescriptive but, instead, describe the latest best practices for capturing and handling bats to promote their welfare during capture-mark-recapture surveys. We provide recommendations related to biosafety; capture and removal from nets and traps; techniques for restraint, handling, holding, and release; methods for short- and long-term marking; collection of biological samples; photography; euthanasia; and health surveillance.

Introduction

Much maligned for centuries, bats are now recognized for their many ecosystem services. For instance, aerial insectivorous species in North America are primary consumers of nocturnal insects and can eat their weight in prey in a single night (Kunz et al. 2011). Bats annually provide between \$54 billion and \$1 trillion United States Dollars in global pest control, thereby reducing the need for pesticides (Frank 2024, Kunz et al. 2011, Ramírez-

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Fráncel et al. 2022). At the same time, bats are among the most threatened animals globally, due to direct and indirect effects of habitat degradation and loss caused by resource exploitation, anthropogenic industries, and development (International Union for Conservation of Nature 2022, Millon et al. 2018, Theobald et al. 2020). The desire to understand, protect, and conserve bats, therefore, has been on the rise in North America, resulting in an increase in monitoring and research of populations.

Compared to the United States (US) and Mexico, species richness in Canada is low, but there are likely millions of individual bats across the country. Twenty-two species have been recorded in Canada (British Columbia Bat Action Team 2024, Canadian Endangered Species Conservation Council 2022, Naughton et al. 2012; Table 1), some of which have only recently been observed in the country and may be vagrant. As the second largest country in the world, in terms of area, Canada provides tens of millions of square kilometers of habitat and diverse ecological conditions. Canada, consequently, provides a significant portion of the range-wide breeding and hibernating habitat for several species. For instance, Canada represents 50% and 40% of the global range of Little Brown Myotis and Northern Myotis, respectively (Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2013). Yet, there are substantial knowledge gaps in our understanding of the diversity, distribution, abundance, natural history, and health of Canadian bats (Jung et al. 2014).

In 2003, the Canadian Council on Animal Care (CCAC) published the *Species-specific recommendations on: BATS* (CCAC 2003) in recognition of the increasing national trend in bat research and a need for standardized best practices. Now, 2 decades later, work with bats on the Canadian landscape has changed. Once-novel survey tools, such as passive integrated transponders (PIT tags), are now used extensively, while improvements to existing tools and practices have also been made. Meanwhile, the arrival and spread of white-nose syndrome (WNS), caused by the fungus *Pseudogymnoascus destructans* (Blehert and Gargas) Minnis and D.L. Lindner (Pd), has resulted in dramatic population declines in Little Brown Myotis, Northern Myotis, and Tricolored Bats (Environment and Climate Change Canada [ECCC] 2018; Segers et al. 2021, 2024). Consequently, these 3 species are now federally listed as endangered in Canada under the Species at Risk Act (SARA) (ECCC 2018). At the same time, expansion of the wind-energy industry poses significant challenges for the long-distance migrants—the Northern Hoary Bat, Eastern Red Bat, and Silver-haired Bat (Arnett and Baerwald 2013). These migratory species were assessed in May 2023 by the Committee on the Status of Endangered Wildlife in Canada and recommended for federal listing as endangered under SARA (COSEWIC 2023). The endangered status of these 6 species has precipitated an even greater demand for bat-related research and monitoring, particularly population censuses and health surveillance (i.e., monitoring and tracking Pd/WNS and rabies; Edwards et al. 2022). This increase in work with bats is also raising concerns for their welfare when being handled (Edwards et al. 2022), particularly with respect to pathogen transmission.

In response to the ever-growing interest in bats and their protection, guidelines for research methodology have appeared in the peer-reviewed and grey literature over the last 2 decades. For instance, existing documents detail procedures for capturing, handling, transporting, holding, captive care, medical procedures, and research-specific techniques (Kunz and Parsons 2009; Lollar 2010, 2018; Sikes et al. 2016). In the US, strict regulations must be followed to obtain permits to capture and handle listed species (US Fish and Wildlife Service [USFWS] 2020). In Canada, however, regulations do not yet exist. One exception is Quebec's mandatory and recommended practices for handling bats (Québec Ministère des Forêts de la Faune et des Parcs 2021). Although Alberta (Vonhof 2006) and British Columbia (Resources Information Standards Committee [RISC] 2022) have produced documents

Table 1. Conservation status and distribution of 22 species of bats recorded in Canada (British Columbia Bat Action Team 2024, Canadian Endangered Species Conservation Council 2022, Naughton et al. 2012). Conservation status as assessed by COSEWIC (2023) and current as of 31 March 2025. Dark shaded cells indicate records of each species in a province or territory (see footnote for coding). No shading means no record.

Species by scientific name, authority (common name)	COSEWIC Status[1]	Province or Territory[2] YT	NT	NU	BC	AB	SK	MB	ON	QC	NB	NS	PE	NL
Family Molossidae														
Nyctinomops macrotis (Gray) (Big Free-tailed Bat)[3]	na				■									
Tadarida brasiliensis (I. Geoffroy) (Brazilian Free-tailed Bat)[3]	na				■									
Family Vespertilionidae														
Antrozous pallidus (LeConte) (Pallid Bat)	TH				■									
Corynorhinus townsendii (Cooper) (Townsend's Big-eared Bat)	na				■									
Eptesicus fuscus (Palisot de Beauvois) (Big Brown Bat)	na	■	■		■	■	■	■	■	■	■			
Euderma maculatum (J.A. Allen) (Spotted Bat)	SC				■									
Lasionycteris noctivagans (Le Conte) (Silver-haired Bat)	EN	■	■		■	■	■	■	■	■	■	■		■
Lasiurus borealis (Müller) (Eastern Red Bat)	EN				■	■	■	■	■	■	■	■		■
Lasiurus cinereus (Palisot de Beauvois) (Hoary Bat)	EN	■	■	■	■	■	■	■	■	■	■	■	■	■
Myotis californicus (Audubon and Bachman) (California Myotis)	na				■									
Myotis ciliolabrum (Merriam) (Western Small-footed Myotis)	na				■	■	■							

[1]EN = endangered; TH = threatened; SC = special concern; DD = data deficient; na = not assessed. [2]YT = Yukon; NT = Northwest Territories; NU = Nunavut; BC = British Columbia; AB = Alberta; SK = Saskatchewan; MB = Manitoba; ON = Ontario; QC = Quebec; NB = New Brunswick; NS = Nova Scotia; PE = Prince Edward Island; NL = Newfoundland and Labrador. [3] Possibly vagrant; residency undetermined (British Colombia Bat Action Team 2024, Canadian Endangered Species Conservation Council 2022).

Table 1. Continued.

Species by scientific name, authority (common name)	COSEWIC Status[1]	Province or Territory[2]												
		YT	NT	NU	BC	AB	SK	MB	ON	QC	NB	NS	PE	NL
Family Vespertilionidae														
Myotis evotis (H. Allen) (Long-eared Myotis)	na													
Myotis leibii (Audubon and Bachman) (Eastern Small-footed Myotis)	na													
Myotis lucifugus (Le Conte) (Little Brown Myotis)	EN													
Myotis septentrionalis (Trouessart) (Northern Myotis)	EN													
Myotis sodalis (Miller and G.M. Allen) (Indiana Myotis)[3]	na													
Myotis thysanodes Miller (Fringed Myotis)	DD													
Myotis volans (H. Allen) (Long-legged Myotis)	na													
Myotis yumanensis (H. Allen) (Yuma Myotis)	na													
Nycticeius humeralis (Rafinesque) (Evening Bat)[3]	na													
Parastrellus hesperus (H. Allen) (Canyon Bat)[3]	na													
Perimyotis subflavus (F. Cuvier) (Tricolored Bat)	EN													

[1]EN = endangered; TH = threatened; SC = special concern; DD = data deficient; na = not assessed. [2]YT = Yukon; NT = Northwest Territories; NU = Nunavut; BC = British Columbia; AB = Alberta; SK = Saskatchewan; MB = Manitoba; ON = Ontario; QC = Quebec; NB = New Brunswick; NS = Nova Scotia; PE = Prince Edward Island; NL = Newfoundland and Labrador. [3] Possibly vagrant; residency undetermined (British Colombia Bat Action Team 2024, Canadian Endangered Species Conservation Council 2022).

detailing survey techniques (e.g., net placement to maximize capture success), these documents rarely mention explicitly how best to ensure the welfare of captured bats. Instead, safe-handling practices are implicit because they are often culturally learned and adapted over time—passed down from mentor to mentee—but this collective wisdom may not be permanently recorded (Jung et al. 2020). Best practices may be provided in an institution's animal-care protocols, but they may not be publicly available. Readily available documentation of best practices for handling bats in Canada is, therefore, needed.

Here, we review and update recommendations on safe capturing, handling, marking, and sampling of bats in Canada. These recommendations are not meant to be prescriptive; our goal is to help investigators develop contingency plans to mitigate stress and prevent injuries to bats. We focus specifically on demographic surveys involving catch-mark-release and monitoring of health in free-ranging bats. We provide examples of products for clarity as needed but, in no way, are these meant as endorsements for a particular product. Work requiring more invasive practices, such as transport, captivity, and veterinary medical techniques, are beyond the scope of this paper. We also highlight unpublished updates to established practices and introduce new methods, with the purpose of providing guidelines and precautions for these techniques. In doing so, we aim to provide new and established bat workers, as well as permitting authorities, better access to best practices to promote welfare, based on current thinking of Canadian experts. Although we make specific references to Canadian species, organizations, and authorities, the guidelines should be useful throughout North America. We acknowledge that some recommendations below may differ from restrictions in various geographical jurisdictions across the US and in other countries. Practitioners should, therefore, always follow protocols and conditions outlined specifically in their permits.

Methods

Our recommendations are based on consensus reached through thoughtful discussion of existing published materials and cultural knowledge that spans decades. We reviewed the published and gray literature, if available. We also held 4 workshops in late 2022 and early 2023 to harness the collective knowledge of the authors, who represent experts and specialists from across Canada. This group of experts, referred to as the Canadian Bat Welfare Working Group (CBWWG), represents diverse backgrounds, including academic, non-governmental organization, government, wildlife health, biological consultant, and animal welfare.

If empirical support does not exist for our recommendations, we draw from our collective experience and cite the source of such information as "CBWWG unpubl. data". We provide guidance for each step involved in a study, ranging from the planning stage through capture, data collection, marking, and release. Additionally, we supply examples of suggested products to help readers, although these suggestions are not exhaustive. We also present guidance on how to recognize poor health and how to address situations after poor health is identified.

Finally, based on our cumulative experience and consensus, we suggest "decision thresholds" for when it is safe to capture bats repeatedly in a particular location, time elapsed between checking traps and nets, and how long to hold animals, all of which depend on species, time of night, environmental conditions, season, and bat condition. More conservative thresholds represent the lowest risk to welfare, based on expert opinion. Less conservative thresholds represent those that come with greater, but reasonable, risk to welfare, but may be warranted to meet project objectives, while also adhering to the "3 Rs" of Russell and Burch (1959): reduction, refinement, and replacement.

Planning

Training

Personnel need to receive first-hand training in capture and handling from an experienced professional. In addition to learning techniques of safe handling and capture, training includes how to recognize normal health and behavior to help personnel quickly identify an animal with potential health problems (see "Poor health"). Training also includes learning to euthanize bats humanely, should the need arise (see "Euthanasia"). A mentor should supervise personnel until they demonstrate proficiency in identifying, capturing, and handling bats, similar to procedures followed by biologists working with birds in Canada. In the US, this level of mentorship is required to obtain permits for handling listed species (US Fish and Wildlife Service 2020). In the absence of suitable mentorship, new investigators may gain experience by volunteering with experts or participating in handling workshops.

Permits and permissions

Depending on the project, techniques used, and the location of the work, multiple permits and permissions are often necessary and these take time to acquire. Also, each species may require special permits and handling precautions, due to different life histories, roosting strategies, and conservation status (Table 2). Hence, investigators should begin planning

Table 2. Species-specific recommendations for holding duration and potential for permit requirements with the conservation status reported by the COSEWIC.

Species	Specific Recommendations	COSEWIC Status
Non-target[1]	Identify species. Collect basic demographic information; record band or PIT-tag ID if present; release immediately, but see other species-specific recommendations.	
Corynorhinus townsendii	Acclimate in holding bag for 15–30 min before further handling.	Not assessed, but a candidate species for assessment as of 4 June 2025.
Lasiurus borealis	May require special handling permits, depending on provincial and territorial Species at Risk legislation.[2] Place in fine-mesh bag; acclimate 15–30 min before further handling (CBWWG unpubl. data); these measures may be particularly helpful when handling males (L. Bishop-Boros, Western EcoSystems Technology, Fort Collins, CO, pers. comm.).	Endangered
Lasiurus cinereus	May require special handling permits, depending on provincial and territorial Species at Risk legislation.[2] Place in fine-mesh bag; acclimate 15–30 min before further handling (CBWWG unpubl. data).	Endangered
Lasionycteris noctivagans	May require special handling permits, depending on provincial and territorial Species at Risk legislation[2].	Endangered
Myotis lucifugus	May require special handling permits, depending on provincial and territorial Species at Risk legislation; Edwards et al. (2022) recommend *M. lucifugus* be released within 30 min of capture.	Endangered
Myotis septentrionalis	May require special handling permits, depending on provincial and territorial Species at Risk legislation.	Endangered
Perimyotis subflavus	May require special handling permits, depending on provincial and territorial Species at Risk legislation.	Endangered

[1]Non-target refers to bat species not part of the project or study and, thus, handling can be avoided or minimized.
[2]At the time of publication, these species were under review for listing as Species at Risk in several jurisdictions.

several months in advance of their proposed start date to allow time to consult and obtain approval from institutional animal care committees, as well as permits from federal, provincial, and territorial wildlife departments. Permits may also be required for project materials or equipment (e.g., mist nets, forearm bands, and drugs for euthanasia). Federal, provincial/territorial, interprovincial/interterritorial, or international permits may be required to ship samples to laboratories for analysis. Personnel should be mindful that they are bound to the sampling procedures, species, and numbers of bats specified in permit approvals.

In addition to obtaining the necessary permits, investigators should obtain permission to access lands where they plan to capture bats, including consultation with Indigenous peoples, if appropriate or required. The Government of Canada provides details about all First Nations and Indigenous peoples found in the country, including their geography and the locations of their reserves (https://fnp-ppn.aadnc-aandc.gc.ca/fnp/Main/index.aspx?lang=eng). If work is to be conducted on private lands, investigators should obtain approval from landowners. Many provinces and territories have online resources to establish property ownership.

Welfare protocols

Investigators should consult the *Animal Behaviour Society's Guidelines for the Use of Animals* (Society for the Study of Animal Behaviour 2022), paying particular attention to the "3 Rs" of reduction, refinement, and replacement (Russel and Burch 1959). Critical thought is needed to determine the minimum sample size required to meet study objectives (reduction), how existing protocols and procedures can be modified to reduce their impact on captured bats (refinement), and if alternative methods can be used to obtain the requisite data (replacement). To minimize the stress experienced by bats, establish *a priori* thresholds for time spent in traps or nets before removal, time to remove bats from traps or nets, handling time during processing, and total holding time from capture until release (Table 3). Consider how each of these thresholds is affected by intrinsic (e.g., species, sex, age, reproductive stage, and health of bats) and extrinsic factors (e.g., region, habitat, time of year, time of night, and environmental conditions; Tables 2–3). Specify predetermined considerations for use of euthanasia. Monitor welfare continuously during surveys so that capture and handling procedures can be modified as needed (i.e., refinement).

Before starting a project, determine if veterinary expertise is available, and review recommended protocols, in case of incidents that harm bats, or if sick or injured individuals are captured. This can be accomplished by consulting medical references specific to bats (e.g., Lollar 2010, 2018) and contacting available experts, including those at local wildlife health centers (e.g., Canadian Wildlife Health Cooperative [CWHC] Regional Centres), as well as licensed wildlife rehabilitators or veterinarians familiar with bats. Provide contact information for experts to all personnel on a project. The Neighbourhood Bat Watch provides a list of wildlife rehabilitation centers in Canada accepting bats (https://batwatch.ca/wildlife-rehabilitation-centres-accepting-bats).

Biosafety

Pathogens

Bats are reservoirs for pathogens infectious to other bats and other wild and domesticated animals, as well as to humans (zoonoses; Dutheil et al. 2021, Joffrin et al. 2018, Wibbelt et al. 2009). Causes, prevalence, symptoms, treatment, and prevention of transmission of pathogens found in bats in Canada are discussed in detail elsewhere in the literature, including the rabies virus *Lyssavirus* genotype 1 (British Columbia Centre for Disease Control 2025; Fenton et al. 2020; Government of Canada [GC] 2015, 2018; Segers et al. 2021; US Centers for Disease Control and Prevention [USCDC] 2024); the fungus *Histoplasma capsulatum* Darling, the cause of histoplasmosis (Ashraf et al. 2020, Canadian Centre for Occupational Health and Safety [CCOHS] 2023, CWHC 2024a, Nicolle et al. 1998); and Pd, the fungal cause of WNS (Blehert 2012; CWHC 2024b, c, d; Shelley et al. 2013).

Like all animals, bats have host-specific viral, bacterial, and fungal pathogens, as well as parasites (Avena et al. 2016, Czenze and Broders 2011, Irving et al. 2021). Preventing transfer of pathogens and parasites among bats during surveys is crucial, even if these organisms

Table 3. Suggested range of temporal thresholds for capture, handling, and holding bats, based on feedback from 8 experts, representing eastern, central, western, and northern Canada. More conservative thresholds represent the lowest risk to bat welfare, based on expert opinion. Less conservative thresholds represent those that come with greater risk to bat welfare, but may be warranted to obtain the data needed to conduct a survey successfully. For details, consult relevant sections of the text. "Basic data" refers to species identification and sex, with age and reproductive status noted, if readily observable.

Category	More conservative	Intermediate	Less conservative
Time between trapping/netting			
Free-flying bats in the same location[1]	7 nights	4–5 nights	1–2 nights
Free-flying bats in same area with nets in different place[1]	5 nights	1 night	0 night
Maternity roost	≤30 nights	5–10 nights	3 nights
Night roost	≤30 nights	5–10 nights	3 nights
In hibernacula	1–2 times/season[3]	30 nights	0–1 night
Free-flying bats outside hibernacula during swarming[2]	1–2 times/season[3]	14 nights	7 nights
Frequency of trap/net checks			
Mist nets	5 min	10–15 min	20–30 min
Mist nets at maternity roosts	Continuous at emergence, then as above		
Harp traps	30 min	1–3 h	1–2 times/night
Harp trap at maternity roosts	Continuous to 15 min at emergence, then 60–90 min thereafter		
Holding times			
Maximum time a bat should be in net	15 min	20 min	30 min
Experienced personnel to remove bats from mist nets	≤5 min	10 min	15 min
Time to get basic morphometric and demographic data	10 min	≤15 min	20 min

[1]I.e., trail, forest edge, etc. [2]Same individuals rarely captured within 1–2 weeks (C. Lausen, Wildlife Conservation Society, Kaslo, BC, Canada, pers. comm.). [3]E.g., at beginning and end of season. [4]Review sections on "Training" and "Poor health". [5]Holding involves temporarily retaining the bat short term, typically in a bag, whereas handling involves personnel having the bat in hand, often to collect morphometric measurements or other biological data. [6]Provision with food and water, especially if adhering to the less conservative thresholds. [7]Depends on factors such as nightly temperature, use of torpor in captured individuals, and body size; facilitation of torpor/warming also depends on these factors. [8]Bat must be provided exogenous heat during the entire holding time so that the animal expends minimal energy on maintaining a normothermic body temperature.

Table 3. Continued.

Category	More conservative	Intermediate	Less conservative
Holding[5] times (warm season or summer)			
Non-target species	Basic data and release immediately	30 min	
Any bat in poor condition[4]	Basic data and release immediately	30 min	
Bats in distress or at risk of morbidity or mortality due to capture	Release immediately		
Target species - individuals in good condition - within first hour of sunset			
Females with attached pup(s)	Basic data and release immediately	30 min	1 h
Late pregnancy females	Basic data and release immediately	30 min	1 h
Nursing females (pups not attached)[6]	1 h		2 h
Early pregnancy females	1 h	2–3 h	4 h
Adult males, nonparous and post-lactating females, volant juveniles	1 h	2 h	4 h
Target species – individuals in good condition - between sunset and sunrise not including 1 h after sunset or 1 h before sunrise			
Females with attached pup(s)	Basic data and release immediately	30 min	1 h
Late pregnancy females	Basic data and release immediately	30 min	1 h
Nursing females (pups not attached)[6]	1 h		2 h
Early pregnancy females	1 h	2–3 h	4 h
Adult males, non-parous and post-lactating females, volant juveniles	1 h	3 h	4 h
Target species - individuals in good condition - within 1 h of sunrise			
Any bats still being held (nets should be closed)	Basic info and release immediately		30 min
Holding times (not summer)			
Spring[7]	≤1 h but see [7]		
Fall[7]	≤1 h but see [7]		
Winter[8]	1 h but see [8]		

[1]I.e., trail, forest edge, etc. [2]Same individuals rarely captured within 1–2 weeks (C. Lausen, Wildlife Conservation Society, Kaslo, BC, Canada, pers. comm.). [3]E.g., at beginning and end of season. [4]Review sections on “Training” and “Poor health”. [5]Holding involves temporarily retaining the bat short term, typically in a bag, whereas handling involves personnel having the bat in hand, often to collect morphometric measurements or other biological data. [6]Provision with food and water, especially if adhering to the less conservative thresholds. [7]Depends on factors such as nightly temperature, use of torpor in captured individuals, and body size; facilitation of torpor/warming also depends on these factors. [8]Bat must be provided exogenous heat during the entire holding time so that the animal expends minimal energy on maintaining a normothermic body temperature.

do not pose a risk to human health. Although bats in Canada are not known to be reservoirs for SARS-CoV-2 or to be susceptible to the virus, we recommend abundant caution (i.e., appropriate personal protective equipment [PPE]), to limit potential reverse transmission from infected humans to bats (Cook et al. 2022, CWHC 2021). Below, we offer biosafety guidelines to prevent exposure to and transfer of infectious agents among bats, as well as between bats and humans. Employers and permitting agencies may have additional, or different, requirements that must be followed.

Vaccinations

Although specific guidelines vary across jurisdictions (e.g., countries, provinces, territories, and states), all new personnel working with bats must have pre-exposure rabies vaccinations (CCAC 2003, Fenton et al. 2024, GC 2015, Vonhof 2006) and demonstrate a sufficiently high protective titer for rabies antibodies (>0.5 IU/ml). Pre-exposure immunization typically involves 2 or 3 separate doses over a span of a month (GC 2015, USCDC 2024). Careful planning, therefore, is needed to ensure that workers can obtain post-vaccination titers before handling bats to confirm that they have a protective antibody titer. Following initial vaccination, an individual's titer should be checked regularly (Fenton et al. 2024). Every 2 years is typical, but the recommended frequency varies by region (USCDC 2024). In Canada, if an individual's titer drops below 0.5 IU/ml, they must receive a booster dose of the vaccine (GC 2015).

If bitten or scratched by a bat, thoroughly wash the affected area with soap and water, and apply an antiseptic. Canada is a large country with many rural and isolated areas where adequate health care may not be readily available. Therefore, a person with a bat bite or scratch should protect their personal health by obtaining medical advice on the appropriate next steps to prevent a potential rabies infection. In Canada, this can involve reporting the incident to a personal physician, the local medical health official (i.e., employee in the provincial or territorial Office of the Chief Medical Health Officer), or the Public Health Agency of Canada. Importantly, advice provided in our monograph is not meant to replace that of medical professionals. Provincial and territorial health agencies offer the most appropriate medical guidance, including post-exposure prophylaxis. Additional doses of rabies vaccinations (i.e., boosters) will likely be recommended for individuals with a protective titer, while persons without previous immunization will require the vaccine and rabies immunoglobulin (Fenton et al. 2024, GC 2015, Public Health Ontario 2017). If a human or domesticated animal is bitten by a bat, the bat should be kept for further examination. If a bat is suspected to be rabid, it is typically euthanized (see "Euthanasia") and submitted for testing (Fenton et al. 2024). All personnel must, therefore, take precautions to avoid bites or scratches so that healthy animals are not unnecessarily killed to protect human health.

Personal protective equipment

Wearing personal protective equipment (PPE) during fieldwork is strongly recommended, due to the potential transfer of known and unknown pathogens from bats to humans and vice versa (Shapiro et al. 2024). Depending on the type of survey being conducted and level of risk, PPE may include gloves, masks, and outer clothing, which are outlined in Shapiro et al. (2024). We briefly discuss the guidelines, but readers should consult the most current sources available. For instance, the CWHC (2024e) provides detailed PPE guidance for WNS on its website, which is updated when new information becomes available.

Gloves. Always wear gloves to prevent bites, scratches, and pathogen transmission (Couper 2016, Fenton et al. 2024). Typically, disposable surgical-type gloves (latex or nitrile) are used (Couper 2016; CWHC 2021, 2024e) and are worn over well-fitted, thick, reusable gloves (e.g., leather, golf, riding, baseball batting, or gardening gloves) (Couper 2016, Hooper and Amelon 2014, Vonhof 2006). Puncture-resistance tests indicate that deer-hide gloves offer a good balance between retaining dexterity and providing personal protection when handling bats weighing <40 g, and split-leather gloves are recommended for bats >40 g that often have strong bite force (Freeman and Lemen 2009). For some people, disposable gloves worn over thick gloves may inhibit dexterity when handling small bats or when manipulating equipment and tools. One solution is to wear only a disposable glove on the dominant hand requiring dexterity, while holding the animal in the non-dominant hand, which is covered by thick, puncture-resistant glove, as well as a disposable glove. Another option is to wear 2 disposable gloves on each hand, which may be the best choice when handling especially small species (<10 g) with weak bites. Wearing a single pair of orthopedic surgery gloves that are equivalent to or greater than the thickness of 2 disposable gloves may be a suitable alternative that provides equivalent protection, but greater dexterity, compared to reusable gloves. When choosing a disposable glove, be aware that bats may be allergic to latex (CWHC 2024e). In addition, take care when using nitrile gloves, because bats can have difficulty freeing their teeth after biting into the nitrile material (CBWWG unpubl. data).

Masks. For masking, we suggest following the latest recommendations related to SARS-CoV-2, because guidelines are often context specific and always evolving (ECCC 2022, Shapiro et al. 2024). Current practice suggests use of respirators with a high-efficiency particulate air (HEPA) filter, capable of removing 2-µm particles, for anyone working in building roosts or caves, where there is a risk of exposure to spores of *H. capsulatum* (CCOHS 2023). When personnel handle bats, respirators without exhalation valves are recommended by CWHC (2021), though it is unknown if respirators help protect bats from potential exhaled human pathogens.

Clothing. Wearing multiple layers of clothing that provide full body coverage can prevent direct contact between an animal and a worker's skin, as well as protect inner clothing from potential contamination by pathogens. Multiple layers are difficult for a bat to bite through and long sleeves that cover the arms to the wrists prevent bites or scratches. Wearing outer clothing, such as coveralls and footwear coverings, is useful because outer layers can be removed and changed before moving between sites, thus reducing transfer of pathogens, like Pd (CCOHS 2023; CWHC 2021, 2024e). Disposable suits, such as Tyvek (Dupont, Wilmington, DE), should be discarded as biohazardous waste, but cloth coveralls can be washed and re-used (see references and links in "Decontamination" for guidance).

Topical lotions and sprays. Avoid exposing bats to sunscreen or insect repellants. After application or touching any treated areas of the body, workers should thoroughly wash their hands with soap and water. Alternatively, lotions and repellants may be applied with bare hands before donning gloves, thereby preventing chemical contact with animals. When applying or reapplying sunscreen or repellants, move away from traps, nets, processing stations, and areas where bats are held.

Decontamination. Decontaminate equipment and materials before moving to another site where individuals from different colonies are likely to be captured. Sterilize all gear that can be submersed in liquid (e.g., nets, traps, PPE, holding bags, and processing materials), and disinfect any surface of delicate or electronic equipment (i.e., headlamps, weighing scales, restraining devices, and cameras) that may have touched bats or roost surfaces. Equipment that has previously contacted bats outside Canada should not be taken into Canada and vice versa. Additionally, it is advisable to have separate equipment and materials to reduce the risk of pathogen spread across regions where pathogens may differ (RISC 2022). However, it may be impractical or unaffordable to have dedicated gear for different regions if study areas span jurisdictional borders, further highlighting the need for thorough decontamination.

Detailed guidelines are available for decontaminating capture equipment, clothing, footwear (CWHC 2024e), and processing materials (Iowa Department of Natural Resources 2016; see Supplemental File 1, available online at http://www.eaglehill.us/NABRonline/suppl-files/nabr-024-Patriquin-s1.pdf, for an overview of decontamination guidelines in different seasons). Although these protocols indicate products that are suitable to use for decontamination, check for updates annually, because recommendations can change. For example, North American guidelines no longer recommend ethanol for disinfecting gear but, instead, suggest isopropanol at concentrations of 50–70% as 1 of several options for a decontamination chemical (CWHC 2024e, White-nose Syndrome Disease Management Working Group 2024).

Capture and Removal from Nets and Traps

General guidelines

Capture of free-flying bats usually involves mist nets or harp traps, but catching bats at roosts may be done directly by hand, with hand nets, or with specialized "roost traps". Nets and harp traps should be clearly marked with signage and reflective tape on the poles when placed in areas frequented by pedestrians, bikes, ATVs, vehicles, or boats. It may be helpful to request homeowners keep cats indoors when placing traps or nets on their properties, because domestic cats may pose a risk to captured animals (de Moura et al. 2023). Avoid capture efforts during poor weather to minimize unnecessary stress on bats. For example, mist nets can billow in windy conditions, which may result in bats becoming severely entangled. Netting or trapping on nights with precipitation can result in wet bats, which have to expend additional energy to keep warm (see "Torpor"). If inclement conditions arise during a capture event, close nets and traps.

Holding time should be minimized to the extent possible (see "Holding duration" for recommendations; Table 3). Holding time refers to the total time a bat is captive, including time in a net or trap, time during removal, time held in bags, and time being processed. Maximum duration of each of these steps should be determined *a priori* and depends on method of capture, location, species, and project objectives (Table 3). For each individual, record the time of capture on tags attached to the holding bag, in a field notebook, or on a datasheet. We recommend setting reminders of approaching times to check traps or nets and of approaching release times (see "Holding duration" and Table 3). Alarms set on timers or cell phones (preferably vibrating) can alert workers that may be preoccupied with removing and handling other bats (see "Holding duration"). Bat detectors (see Supplemental File 2, available online at , available online at http://www.eaglehill.us/NABRonline/suppl-files/nabr-024-Patriquin-s2.pdf) set near traps or nets and within hearing range of field personnel can provide ongoing feedback on activity, which may help determine the frequency of checks and appropriate timing of closure. Baby monitors also help detect captured bats, because the animals often emit audible vocalizations when distressed. Bells attached to nets may also signal when a bat has been captured. However, even when there is little or no acoustic activity (ultrasonic or audible), check traps and nets according to the guidelines below; some bats produce calls that are low in intensity or very high in frequency, making the sounds difficult to detect (Fenton 2013).

An adequate number of personnel should be available to remove bats quickly and safely from nets and traps, to process the animals within recommended timelines (Table 3), and to ensure human safety. We suggest a minimum of 2 people, but more may be required, depending on experience, type of net or trap, number of nets or traps, distance between nets or traps, and number of anticipated captures, as well as the data and samples to be collected. Three or more personnel may enable assistance with lowering complex netting systems (e.g., when both ends of the net must be lowered simultaneously) and may expedite processing and any necessary decontamination. The number of workers must also be carefully considered when catching bats at roosts of colonial species, because a large number of captures may occur within a short period at emergence.

Repeated captures may be detrimental for foraging and roosting bats and lead to changes in behavior. For example, free-flying bats may avoid an area if nets are detected (Kunz and Brock 1975, Winhold and Kurta 2008) or individuals are repeatedly captured (Marques et al. 2013). Therefore, capture attempts at a given site should be separated temporally (Table 3) or spatially (e.g., different flyways or bodies of water; RISC 2022). Although some evidence suggests bats continue to use the same roost following capture (Ferrara and Leberg 2005), other bats may delay emergence (Ancillotto et al. 2019) or switch roosts (Lewis 1995, Luo et al. 2012). The costs of disturbing roosts to survival and reproductive success are unknown, but a good precaution is to separate visits by days or weeks (Table 3), particularly to maternity roosts. Alternatively, if the goal is to assess roost occupancy, visual emergence counts or passive monitoring with bat detectors or cameras may be more appropriate options compared to capturing animals (Ahlberg et al. 2025, Eddington et al. 2025, Froidevaux et al. 2020, Jaffe et al. 2024).

Personnel experience

When estimating the number of personnel needed, consider their experience and establish predetermined timelines for closing nets and traps and releasing bats. Generally, experienced individuals can extract a bat from a mist net in ≤5 min, whereas inexperienced personnel often are slower (about double the time for an expert) and more cautious. To become competent, an individual developing their skillset needs oversight and assistance from experienced investigators, especially if intervention is needed when handling time is nearing the recommended maximum (Table 3). Mentor-mentee relationships are paramount for those new to bat work, and mentors should intervene if bat welfare appears to be compromised.

Mist nets

Mist nets were brought to North America about 75 years ago and have become the most widely used instrument to catch free-flying bats, especially in summer (Genoways et al. 2020). Deploying mist nets takes time, especially for inexperienced workers. Therefore, consider the time required for deployment when selecting the number of nets to place in an area and when determining the time to begin deployment. To minimize captures of crepuscular birds, mist nets should remain closed until shortly after dusk, with a piece of string or flagging tape tied around tiers to gather all trammels at several points along the length of the net. Even so, on occasion, personnel should be prepared to release nocturnal and crepuscular animals from mist-nests, including owls and flying squirrels that require thick gloves for removal. If weather conditions become unfavorable (i.e., high winds or precipitation), close nets. Workers should take precautions to avoid becoming tangled in nets, because entanglement may increase tension on the net and cause injury to bats. Consequently, avoid wearing watches, jewelry, or clothing with buttons that can easily be snagged, and when checking nets and removing bats, avoid crawling or bending under nets to reach the other side.

Frequency of net checks depends on various factors. While US permits require nets to be checked every 10 min (https://www.fws.gov/sites/default/files/documents/2024-10/2024_usfws_rangewide_ibat-nleb_survey_guidelines.pdf), no such restrictions yet exist in Canada. Because capture success is typically low across much of Canada (see "Number of Bats below), investigators face a challenge of balancing project objectives against animal welfare. Therefore, depending on objectives, we recommend checking open mist nets every 5–30 min to minimize capture stress (Table 3), risk of predation, or injury (Bunt et al. 2021, Edwards et al. 2022, Lefevre 2005, McAlpine et al. 2011, RISC 2022, Sikes et al. 2016). Various vertebrates, including deer, frogs, owls, and fish, attempt to prey on bats in mist nets (de Moura et al. 2023, Jung et al. 2011), and frequent checks should reduce predation of captured individuals. Checking too frequently (e.g., every few minutes) may deter bats from approaching the net, while infrequent checks could lead to an unmanageable number of captures, thereby prolonging the time each animal spends in the net. Compared to more regular checks, infrequent net checks can also lead to greater entanglement, resulting in increased removal times and stress, and risk significant damage to the nets from bats chewing holes while attempting to escape. Nets should be continuously monitored when positioned near maternity roosts, where many bats may be caught in a short period. Personnel should also check for escaped bats on the ground below and near nets. Furthermore, frequently check nets placed just above a pond or stream to prevent risk of bats drowning (RISC 2022); although bats can swim (Craft et al. 1958), the weight of an entrapped animal may cause the net to sag into the water.

The number of mist nets that can be safely deployed depends on the number of people available, anticipated time to remove bats (see "Personnel experience"), and expected capture rate (see "Number of anticipated bats"). If some personnel have little or no experience, we recommend limiting the number of nets until workers become proficient at quickly freeing bats. Otherwise, it is common practice to deploy as many mist nets as can be checked within 5–30 min, including travel time between nets and time to remove bats (RISC 2022). The type of mist net should also be considered when determining how many can be safely deployed, as well as how often to check nets. Modern mist nets are made of either polyester (e.g., Avinet, Portland, ME) or nylon, which may be a thin monofilament (e.g., Ecotone, Gdynia, Poland) or braided (e.g., Avinet). Anecdotal observations suggest monofilament nets may provide higher capture success, because they presumably are not as easily detected by bats compared to polyester nets (CBWWG unpubl. data). However, bats also appear to become more entangled in monofilament nets, making removal more difficult compared to polyester nets. A comparative study in Africa, though, found no difference in capture success of echolocating bats when using monofilament nylon or polyester mist nets; instead mist nets with small mesh size had higher capture success compared to nets with large mesh (Ferreira et al. 2021). Additionally, some investigators have noted that certain species (e.g., Little Brown Myotis) become less tangled in and easier to extract from monofilament compared to polyester nets (J. Wilson, Government of Northwest Territories, Yellowknife, NT, Canada, 2024 pers. comm.). Because outcomes appear to vary, we recommend carefully monitoring nets of all types initially to determine outcomes for species in the project area. Based on these observations, personnel can adjust number of nets and frequency of checks accordingly, within the recommended guidelines (Table 3).

If many bats are captured in a single net, remove individuals from the lowest tier first, and close each tier as it is freed of bats, until the entire net is cleared. For each tier of a net, we recommend beginning with the least tangled bats, especially if workers are inexperienced, to reduce further entanglement and ultimately decrease handling times. However, give priority for immediate removal to juveniles, individuals at risk of injury or that appear distressed, or females that are obviously pregnant, and then to target species. Lower priority may be given to bats that are heavily entangled and especially difficult to remove, if they do not appear to be in distress, so that less tangled bats can be freed quickly, thereby reducing the total number of bats experiencing prolonged handling time.

Removing bats from upper tiers can be challenging. There are pulley systems commercially available (e.g., BCM Triple High Mist Net Pole System, Bat Conservation & Management, Carlisle, PA; https://www.youtube.com/watch?v=MPuqIst_My0) to lower nets safely and quickly. Alternatively, ropes can be used to create a homemade pulley system (e.g., https://www.youtube.com/watch?v=bL-T2lwtb5g; Kunz and Kurta 1988). Although a stepladder may be used, extreme caution must be taken to prevent placing additional net tension on captured bats that could lead to injury. Care should also be taken to limit entanglement of the ladder in the net. As a last resort and with extreme caution, it may be possible to pull one or both pole(s) out of the ground and tilt the net so that another person can reach the tiers.

Intervention may be required if bats are in distress, severely tangled, or maximum time in the net or trap is approaching. Small scissors or a seam ripper, with its sharp tips dulled, can be used to cut net strands and release a bat in a timely manner. Personnel should be prepared to cut nets when approaching the recommended maximum extraction time or time in net (Table 3).

Bats often bite and hold on to nets and gloves. When this happens, do not react by pulling back or making sudden moves that may injure the bat's body or break teeth. In addition, do not forcefully extract the captured animal by pulling on its scruff or muzzle. A common technique used in the past to encourage a bat to open its mouth and release a net or glove was to blow on the animal's face. However, this practice has generated concern, due to the possibility of SARS CoV-2 zooanthroponosis. One potential alternative to blowing on bats is to remove net tension or loosen grip slightly to reduce strain on bats and promote a relaxed position. A second technique is to spray compressed air from a commercial cannister (e.g., those used to remove dust from computers) on a bat's face; however, first conduct a test spray to gauge distance, force, and direction of spray, and confirm there is no liquid being expelled. Correct use of an air cannister includes using it upright, using short bursts, and not shaking it. A short informative video on how to use compressed air cans correctly can be found online (https://www.youtube.com/watch?v=kPFJNYo_Ka8). A third option to encourage release is to compress silicone bulbs used for cleaning camera equipment (e.g., Soft Tip Silicone Super Air Blower, Jinjiacheng Photography Equipment, Shenzhen, China), thereby producing a burst of air onto the bat's face. Gently tapping a bat on the head also may cause the animal to open its mouth. Another technique is to use a thumb or forefinger to apply gentle pressure on top of a bat's head, while sliding the thumb/forefinger to the base of the neck; these maneuvers tilt the head back and cause the bat to open its mouth and release its grip (Fig. 1). As a last resort, open a bat's mouth gently using non-metallic forceps, a cotton swab, or similar object (Hoffmann et al. 2010).

When workers become overwhelmed by the number of captured bats, immediately close empty nets by collapsing and tying all tiers together. Tiers should never be closed if they contain bats, because they will likely become enmeshed in multiple tiers. If processing times may exceed maximum predetermined thresholds (Table 3; see "Restraint, Handling, and Release"), nets should remain closed until personnel can remove and process the most recent captures. We advise closing all nets 30–60 min before dawn, to allow time for removal and processing, so that bats can be released before sunrise; doing so also helps prevent capture of crepuscular birds.

Take great care when removing bats from nets. Limit net tension and carefully remove strands of net from the wings to avoid breaking delicate finger and arm bones. General tips for removing bats from mist nets are provided in Supplemental File 1. A video that demonstrates removal of a bat from a net is also available (https://www.youtube.com/watch?v=iY9BNeVk3xs).

Harp traps

Harp traps are recommended at sites where many bats could be captured in a short period, such as at a maternity roost or hibernaculum, and may also be useful for capturing bats in narrow flyways (Tanshi and Kingston 2021). Monitoring of harp traps at maternity roosts and hibernacula should be continuous up to 15 min after initial emergence concludes, and then traps can be checked every 60–90 min. In areas with low activity and low predation risk, harp traps can be inspected every 1–3 h or, in some cases, as little as twice per night. In practice, however, traps should be checked every 30 min to limit holding times and prevent overcrowding (USFWS 2024; Table 3), which can lead to fighting and increased potential for injuries (Wilson 2016). Squabbling bats may also produce audible vocalizations that attract predators or inquisitive humans. Furthermore, overcrowding can increase transmission of parasites and pathogens between individuals that may otherwise not come into contact, such as those of different species or those originating from different colonies. Although the interior of most trap bags is partly covered with a plastic lining, under which trapped bats generally roost, regular checks are also important to prevent bats from getting cold or wet if weather becomes inclement.

Precautions and considerations, similar to those described for mist nets, should be applied to harp traps. For instance, reduce intervals between trap checks when females may

Figure 1. To encourage the bat to release its grip and to examine tooth wear, carefully slide thumb from top of the bat's head to the base of the neck, causing the head to tilt back (Photo by Krista Patriquin).

be in late pregnancy or nursing. The number of traps that can be safely deployed depends on how frequently they are checked, and personnel should consider travel time between traps, anticipated capture rate (see “Number of anticipated bats”), and handling and processing times. If total anticipated processing time exceeds the total recommended holding time (Table 3), remove harp traps from the flight path or lay traps flat on the ground, until most captured bats are processed and released. Simply removing the holding bag of the trap also prevents captures. However, bats may hit a still-erect trap and fall to the ground, and grounded bats can have difficulty taking flight and could get trampled or predated.

One main advantage of harp traps is that workers can remove bats quickly and easily. Nevertheless, take care not to pull bats from the holding bag by their forearms or toes. When grasping bats, check that the wings are closed and the nails are not hooked into the fabric of the holding bag. Like with mist nets, consider time in traps and processing time to determine when to release bats (see “Holding duration”). In areas with a high capture rate, we recommend replacing or disinfecting harp trap bags (and drying them before reuse) periodically throughout the night, to minimize potential transmission of pathogens and parasites among captured individuals. However, disinfection is not required if working at a single roost, since pathogens and parasites will likely spread naturally among group members.

Hand nets

Hand nets with solid cloth or fine nylon-mesh fabric, such as insect sweep nets, may be used to capture bats from walls or ceilings in roosts. To prevent bats from escaping, place a plastic collar around the inner perimeter of the net, similar to that found in the bag of a harp trap. To catch bats as they roost, approach the animals from below and quickly cover them with the hand net, but take care not to pin bats between the metal rim of the net and the roosting surface; bats should fall or fly into the net. Pinch the top of the net to prevent escape with 1 hand; use the other hand to reach through the opening and remove the animal. Be sure that the wings are folded and thumbs and toes are carefully unhooked, rather than pulled, from the net fabric (Finnemore and Richardson 2004). Use of hand nets to catch free-flying individuals is discouraged because animals may be injured if they collide with the stiff rim at the opening of the net (CCAC 2003, Jackson 2003). If you must catch an animal that is in flight, approach the flying bat from behind rather than head on (CCAC 2003). If possible, conduct captures at roosts during the day, when bats are likely at rest, possibly torpid, and, therefore, easier to catch. Process captured individuals as quickly as possible. If data collection occurs early in the day, release the bats directly inside the roost once processing is complete. However, if processing occurs late in the day, bats can be held in bags or bins (see “Holding bags and bins”) and released after dark, outside the roost.

Roost traps (bag traps)

Roost traps (also called bag traps) are home-made and designed to capture bats as they exit roosts (Kunz and Kurta 1988; e.g., Fig. 2). Roost traps resemble a small, modified harp-trap and bag and can be held in place by hand or attached to the roost. Roost traps may also be fastened to a pole and raised to the roost exit. To prevent bats from escaping or being crushed, make sure the trap makes a tight seal around the roost exit to individuals from trying to escape. Position the trap at least 30 min before dusk and remain quiet while waiting for bats to emerge; otherwise, the animals may perceive a threat and delay departure (Ancillotto et al. 2019). Remove the trap within 1 h after dusk if bats do not emerge, so they can leave to feed. Alternatively, if traps are held in place by hand, traps can be removed and then replaced over the opening once bats begin to leave.

Figure 2. Home-made roost trap. Photo by Krista Patriquin.

Hand capture

When accessible, bats in roosts may be captured directly by hand (CCAC 2003). To extract a bat from a crevice, gently pull the forearm until the animal starts to move in the desired direction, then gradually continue to pull the animal closer until removal is successful. Bats in tight crevices can be extracted with long tissue forceps that are blunt and padded (Sikes et al. 2016). Take care to avoid causing bruises, abrasions, or broken bones, and cease extraction if the animal cannot be removed with relative ease.

Number of anticipated bats

The number of bats that might be captured during a sampling period will inform the sampling effort required to meet project objectives, as well as the the number of personnel required to handle bats safely and quickly. Predicting how many bats may be captured at any given time is challenging, especially during the first site visit. In general, nightly activity and captures vary with geographic region, climate, season, weather, and habitat. Information about capture rates may be found by examining peer-reviewed and grey literature, as well as by consulting colleagues, who may have worked in the general region. To gain insight to potential capture rates at roosts, conduct emergence counts on preceding nights. Acoustic monitoring provides a rough sense of local activity, which may help predict the capture success of free-flying bats.

Geographic region and climate. Compared to the equatorial region and low elevations, fewer species of bats and fewer individuals occur at northern latitudes and high altitudes, because night-time temperatures are often sub-optimal for bats, even in summer (Alves et al. 2018). Bat populations are also reduced in regions with endemic WNS, such as eastern Canada (Balzer et al. 2021, ECCC 2018). In areas where few species or individuals are expected, a high level of effort (e.g., increased number of mist nets, nights, and hours) may be needed to capture enough animals to meet study objectives. However, as always, consider total holding and handling time, and ensure sufficient personnel are available to check nets and traps regularly, as well as to remove and process bats efficiently (see "Holding duration").

Weather and season. Bat activity in any region depends on local weather and season. Factors that affect flight, prey availability, and thermoregulation influence the diversity and number of individuals that are active on any night. Most insectivorous species do not typically forage at air temperatures <10 °C, because few insects are flying (Wolbert et al. 2014). At high elevations or in the far north, bats may remain active at cooler temperatures than at lower elevations or latitudes (e.g., Luszcz and Barclay 2016, Thomas and Jung 2019). In addition, species like Little Brown Myotis, Northern Myotis, Long-eared Myotis, and Pallid Bat can glean prey from vegetation (Norberg and Rayner 1987), and at least some appear to do so at cool temperatures (e.g., <10 °C), when flight of aerial insects ceases (Chruszcz and Barclay 2003, Maucieri and Barclay 2021). Bat activity decreases with wind speed and precipitation (Erickson and West 2002, Gorman et al. 2021, Wolcott and Vulinec 2012), because wind and rain also reduce the number of aerial insects, may interfere with echolocation (Griffin 1971, Voigt et al. 2011), and increase the energetic cost of flight (Voigt et al. 2011). Weather conditions should, therefore, be considered when designing bat surveys.

Bats in Canada are most active in summer, and either hibernate from mid-autumn to early spring or migrate south. The timing of hibernation and migration, however, depends greatly on local climate and can vary from year to year. Investigators should, therefore, consult local experts and climate data to establish the approximate timing of hibernation and migration. Bats occasionally fly during winter, especially inside and near hibernacula, due to changes in ambient temperature, dehydration, disturbance, or in response to WNS (Hoyt et al. 2021). Evidence suggests bats are also flying in winter, away from hibernacula, at temperatures as low as -8 °C (Klüg-Baerwald et al. 2024, Lausen et al. 2022). Therefore, weather and season should be considered when determining the appropriate level of effort required to meet project objectives, while also ensuring animal welfare (see "Season"). However, see precautions associated with capturing bats in winter in "Holding duration, Season".

Habitat. Bat activity also depends on habitat (e.g., forest type, grasslands, waterbodies, etc.), but habitat use varies considerably across species and demographically within species (Lintott et al. 2014). Nevertheless, a few key trends are consistent across species. For instance, activity is often high over ponds and other calm bodies of water, including puddles for maneuverable species, because bats typically drink after evening emergence from their roosts (Ancillotto et al. 2019, Broders et al. 2003). Also, the diet of Canadian bats includes adult stages of many types of insects that emerge from, and swarm over, bodies of water (Clare et al. 2011). In summer, maternity roosts and surrounding areas frequently yield a higher capture rate compared to flyways used by foraging or commuting bats. Similarly, hibernacula and surrounding areas may be hot spots for activity, with bats of some species aggregating (i.e., swarming) to mate before hibernation begins (Randall and Broders 2014). When you are assessing the necessary effort to achieve project goals, it is imperative to take habitat into account while also prioritizing the welfare of bats.

Restraint, Handling, and Release

Bats in hand

Various techniques can be used to restrain bats, but in all cases, gloves should be worn (see "Personal protective equipment, Gloves"). A common way to grasp a bat, known as the "Nelson hold", allows easy transfer of the animal between personnel by placing an index finger between the bat's scapulae, and using the thumb and middle finger of the same hand to hold the animal's forearms against the sides of the body (Fig. 3). Make sure forearms are not extended behind the back, which may strain wing muscles. Another technique, the "Palm grasp/hold" permits easy manipulation of a bat for inspection, measuring, and sampling. Gently place the index finger (or thumb) of the non-dominant hand under the jaw, and the thumb (or index finger) on top of the neck, and gently grasp the animal's body in the palm (Fig. 4). Lightly pressing the digit on the bat's head keeps the jaws closed and prevents biting. Using the Palm grasp with the nondominant hand and carefully adjusting the grip allows easy exposure of specific anatomical areas for examination and measurement with the dominant hand (Fig. 4). To further secure a bat, it can be wrapped in a bag and gripped in the palm, exposing the area of the animal to be measured or sampled. Never hold bats solely by wing tips, thumbs, or forearms because a bat struggling to escape while in this hold can damage flight muscles and break bones (Fig. 5; Bat World Sanctuary 2024).

Holding bags and bins

When researchers are holding bats for processing, they often are placed in a "bat bag" with a drawstring (e.g., 20-by-30-cm, cotton, drawstring bags) (Vonhof 2006). Bags can be purchased from various suppliers (Supplemental File 2) or easily made. Drawstrings provide a tight initial closure, and tying the exposed drawstring around the cinched top is the best method to prevent bats from escaping (Fig. 6). A clothes pin placed beneath the cinched opening provides additional security to prevent escape (Fig. 6). While securing the drawstring, make sure the bat is in the bottom of the bag, so that the animal is not accidentally crushed or tied up.

Figure 3. Proper handling technique, called the "Nelson hold". Wings are held at the side of the bat's body, which prevents over-extending the arms if the bat were improperly held by pinning the wings back. Photo by Jared Hobbs.

Figure 4. Recommended holding technique, called the "palm grasp" (top––photo by Jordi Segers), which is useful during examination (bottom left—photo by Bob Brett) and measuring (bottom right––photo by Jason Headley).

Figure 5. Improper holding technique, with outstretched wings held by bare fingertips. Photo by Florent Valetti; photo taken before widespread recommendation to use gloves for holding bats.

Figure 6. Bags for holding bats with drawstring tied around cinched top. Left: Mesh bat bag. Photo by Cori Lausen. Right: Cloth bag; note plastic clothespin that may be added to prevent escape and wooden clothespin that can be used to label bags. Photo by Jordi Segers.

The most appropriate holding bag differs across species. We recommend cloth bags for crevice-roosting bats (i.e., most Canadian bat species) that are accustomed to roosting in sheltered, confined spaces (Fig. 6). Foliage-roosting bats like Northern Hoary Bat and Eastern Red Bat appear less stressed and less susceptible to capture myopathy when held in fine-mesh "produce" bags, compared to cloth bags (Fig. 6; Table 2; CBWWG unpubl. data, Jung et al. 2002). To prevent the bat's teeth, feet, or thumbs from becoming entangled with threads, avoid bags with frayed seams or loose threads, or turn bags inside out so that seam edges are not inside the bag. After bats are released (see "Releasing bats"), cloth bags can be turned inside out, shaken to remove guano or parasites, and decontaminated before reuse (see "Decontamination"). Although disposable paper bags minimize transmission of pathogens and parasites, bats may not be able to cling to the smooth surfaces, and noise, including ultrasonic sounds, from crinkling paper bags may further stress bats. In addition, some bats may quickly chew holes in paper bags, increasing the risk of escape. Paper bags lined with plastic mesh may provide a suitable alternative, but never hold live bats in bags made of non-breathable material, such as plastic grocery bags.

To facilitate record keeping, mark bags with unique identifiers, such as numbers, patterns, colors, or letters. Unique identifiers on bags can help track the number of captured bats, capture times, species, and demographic data (if these can be assessed quickly at time of removal from a net or trap). Bags should not be placed on the ground but should be carefully hung near the capture site, preferably away from light, noise, and potential predators (e.g., rodents, carnivores, and raptors). Bags can be suspended from nearby objects (e.g., tree branches) with carabiners or clothes pins (ideally plastic to ease disinfection), or by looping the drawstrings around an object. Bags can also be attached to a spare net pole, length of wire, metal rod, or PVC pipe (i.e., any material that is easily and quickly disinfected) that is laid horizontally across a raised surface, such as a table, workbench, or suspended between 2 trees. A rope or piece of wood may also be used, but their surfaces are porous, cannot be quickly disinfected (see "Decontamination"), and may require prolonged drying times after decontamination. Whatever technique is used, make sure bags are suspended and not at risk of being accidentally dislodged and crushed by personnel. Alternatively, bags could be hung from a piece of wire, rod, or pipe inserted between drilled holes on either side of a plastic bin (Fig. 7). Drill additional holes in the bin for air flow, if the lid is going to be placed on top.

Providing external sources of heat for bats may be advisable, if working at temperatures that might cause bats to go torpid. The temperature at which bats become torpid depends on species, sex, age, reproductive condition, and season, as well as how many bats are held together (see "Torpor"). Generally, though, bats enter torpor at ambient temperatures between 7 and 17 °C (Hamilton and Barclay 1994, Matheson et al. 2010). To prevent torpor, a warm water bottle or heat pack (e.g., hand warmers) can be placed in a thick wool sock or fabric bag at the bottom of a holding bin to provide warmth (Fig. 7). The bin should be of sufficient size to prevent direct contact between the bags and heat source, to avoid burning or overheating the animals. If a water bottle or heat pack is not available, bats can be held in appropriately gloved, cupped hands for rewarming.

Do not hold multiple species together in the same bag and, ideally, only place 1 bat in each bag to prevent transmission of pathogens and parasites between individuals, as well as to minimize stress and potential injuries from fighting (Edwards et al. 2022). A female caught with attached offspring should be released immediately. However, if data specific to breeding females or young are needed, place the female and pups in a bag together. If groups of bats are held together, make sure they are not crowded. A common device with

Figure 7. Holding bin, with bags hung on rod and heat pack in a sock on bottom. Photo by Jordi Segers.

the capacity to hold numerous individuals together is the Myers bag, which consists of a nylon net attached to a metal or plastic receptacle, such as a minnow bucket, trash bucket, or polystyrene (Styrofoam) container (Kunz and Kurta 1988).

Do not leave captive animals unattended in enclosed spaces (e.g., vehicles or motel rooms), where the bats might become lost or injured, or might bite a person, if they escape the holding bag. Ideally, do not reuse bags on the same night. However, if necessary to do so, turn the bags inside out and shake them to remove guano and parasites before reuse; also, be sure that bags are not urine-soaked and only reused for individuals from the same colony. Always disinfect bags between nights (see "Decontamination").

Restraining devices

A restraining device can be useful for taking measurements (e.g., wing morphometrics), applying devices (e.g., radio tags, PIT tags), and obtaining samples (e.g., punch biopsies). The McMaster bat restrainer is effective for handling large species, like the Big Brown Bat, and can easily be modified for smaller species (Ceballos-Vasquez et al. 2014). In addition to minimizing sudden bat movements that could result in injury, this device allows a single person to restrain and conduct procedures on a bat that otherwise would require 2 people.

Holding duration

General guidelines. Holding duration includes time bats spend in nets or traps, being handled during removal from nets or traps, in holding bags, and being processed. Determine appropriate handling and holding times using the 3 Rs (see "Methods" and "Welfare protocols"), together with guidelines provided in Table 3. Bats in poor condition or at risk of mortality due to prolonged capture and handling should be prioritized for immediate release (Table 3). Prioritizing the removal of study subjects from nets and releasing recaptured individuals can also minimize holding time. Quickly assess general health (e.g., check that no injuries have resulted from handling), and release bats recaptured on the same night. Recaptures can be identified by checking for PIT tags, bands, hair clippings, biopsy marks, or other identifiers (see "Marking"). If recaptured within the same season, general assessment of health (e.g., body mass), development (e.g., juvenile development), and reproductive stage can be quickly accomplished before the bat's release. If recaptured in subsequent years, obtaining the full suite of measures (e.g., biometrics, demographics, or biological samples) may be warranted.

Once a bat is removed from a net or trap, experienced personnel can normally collect basic morphometric and demographic data in ≤10 min. However, less-experienced individuals may take longer to accomplish these simple procedures and require oversight to ensure maximum holding times are not exceeded (e.g., ≤15 min intermediate threshold; Table 3). If additional procedures are required, such as marking bats (see "Marking") and collecting biological samples (see "Biological Samples"), longer handling times (e.g., 20 min less conservative threshold; Table 3) may be required. Occasionally, prolonged holding or handling of an individual may be needed to collect data specified in a research protocol and could be preferred over repeated capture events, as per the 3 Rs. If capturing bats from a colonial roost, protracted holding or handling of an individual in 1 event may be less stressful than the cumulative stress of multiple recaptures. Additionally, prolonged holding of 1 or a few individuals to obtain required data may reduce the need for multiple subsequent visits, which would cause cumulative stress to a colony or population.

Considering the above, hold bats for the minimal amount of time required to collect necessary data, and release the animal as soon as possible. Some species can be processed immediately, while others may benefit from being kept in a holding bag in a quiet location for 15–30 min before processing to reduce stress (Table 2). Bats should not be held longer than 4 h, including time in net or trap, acclimation in holding bags, wait duration, and processing (but see “Time of night”, “Local environmental conditions”, “Season”; Table 3). When held longer than 2–3 h, consider provisioning bats with water and food during the holding period or before their release (see “Provisioning bats”). Maximum holding time may vary with time of night, body condition, local environmental conditions, season, species, sex, age, reproductive condition, and torpor (Table 3), as well as permitted research activities and objectives. In addition to the guidelines outlined below, consult previously successful protocols and work with experienced practitioners to verify which bats should be prioritized for handling and release.

Time of night. Time of night when bats are captured should be considered when deciding appropriate holding and release times, so consider sunset and sunrise times, which varies seasonally and with latitude. Sufficient time must be available to process and release bats before sunrise. Generally, bats captured anytime between 1 h after sunset and 1 h before sunrise can be held according to the “General guidelines”. If captured within the first hour of sunset, hold animals for no more than 2 h to ensure there is opportunity for them to feed and drink after release, as foraging activity tends to peak within the first few hours of sunset (Broders et al. 2003). Regardless of time of night, provide food and water (see “Provisioning bats”) if individuals are held longer than 2 h or released near sunrise. If bats are still being held within 1 h of sunrise, release them immediately so they can return to their roost without risking exposure to diurnal predators.

Local environmental conditions. Environmental conditions influence the potential risks associated with, and the likelihood of, catching bats, as well as appropriate holding and release times. Although capturing free-flying bats typically does not take place on nights with inclement weather, environmental conditions in some areas can change quickly (see “Weather and season”). Therefore, be prepared for inclement weather and be mindful of local weather forecasts and patterns. Bats should be released before temperatures fall below 10 °C because prey availability is reduced at these lower temperatures (Wolbert et al. 2014). Local environmental conditions influence whether bats enter torpor, and the additional time required for rewarming them must be considered when determining maximum holding time (see “Torpor” and “Holding bats and bins”). An important exception, though, is when free-flying bats are captured in winter, because they typically do not enter torpor during holding (CBWWG unpubl. data). Generally, bats should not be caught in winter because doing so can lead to over-utilization of the energy reserves required to survive hibernation. If captured in winter, animals should be kept warm to reduce the amount of stored fat expended during captivity (see “Holding Duration, Season”).

If rain begins, decide whether to release bats immediately or hold them until inclement weather passes. Thus, keep track of weather forecasts and avoid capture on nights of high likelihood of even moderate rainfall. Releasing bats in light rain is often advisable over holding them, because they can return to their roosts or take shelter in a nearby night roost. If rain is intense, do not release bats until the precipitation has lessened, because the animals may be unable to fly or navigate well in heavy rainfall (Voigt et al. 2011). If kept, hold bats in a dry area until rain subsides, and rewarm the animals, if necessary, before setting them free (see “Torpor”). Similarly, hold any individuals that may have become wet to allow them to rewarm before letting them go.

Monitor wind speeds to mitigate potential injury to bats entangled in billowing nets. If wind speed increases suddenly, close nets and traps, and consider holding any animals until speeds are lower. Holding the bats reduces their risk of being blown off course and minimizes the energy required to overcome strong winds.

Season. Consider seasonal differences in metabolic demands when establishing appropriate handling and holding times. In spring and fall, night-time temperature is lower, and bats may be more likely to enter torpor compared to summer. Additionally, a bat's energy budget is constrained in spring and fall, as the animal attempts to recoup energy reserves lost during hibernation, or build energy reserves to enter hibernation (Hranac et al. 2021). During summer, females also face the additional energetic demands of pregnancy and lactation. Therefore, minimize added energetic stress by reducing holding times (Table 3) and provisioning bats during these periods (see "Provisioning bats").

Capturing and handling bats in winter is generally not recommended, unless investigators have experience working during this critically sensitive period, or intend to work with experienced personnel. Bats depend on stored energy over the winter, so any event that increases the use of these reserves could impact overwinter survival, because insect prey are unavailable during that time. If capture during winter is required for project objectives, investigators should consult with local experts and the literature, and consider the guidelines below.

Handling and holding recommendations during winter depend on if bats are captured while hibernating, flying in or near hibernacula, or flying on the landscape away from hibernacula. Disturbing bats during hibernation is energetically costly for them—the energy expenditure required to maintain normothermia at typical hibernaculum temperatures may be up to 400 times greater than that necessary during summer torpor (Thomas et al. 1990). Capturing and handling bats during hibernation, therefore, puts them at considerable risk of exhausting their energy reserves. If hibernating bats are captured, holding times should be limited to 1 h to minimize energy expenditure, and torpid animals should be allowed to remain torpid. Active animals should be allowed to go torpid, but if they remain active, they should be kept warm (see "Holding bags and bins").

Bats may be captured flying in or near hibernacula or on the landscape away from hibernacula. Bats arouse naturally during hibernation due to changes in ambient temperature and disturbance, as well as physical, metabolic, and physiologic needs (Boyles et al. 2006), or when affected by WNS (Blehert et al. 2011, Lilley et al. 2016). For bats captured in winter and not exhibiting clinical signs of WNS (see "Poor health"), keep holding times to <1 h and allow animals to go torpid. In areas where bats are naturally active outside hibernacula in winter (e.g., the Canadian Prairies; Lausen and Barclay 2006, Lausen et al. 2022), individuals may not readily enter torpor after capture, despite cold ambient temperatures (CBWWG unpubl. data.). These animals should, therefore, be kept warm (see "Holding bags and bins" for recommendations). Unless project objectives focus specifically on monitoring WNS, individuals exhibiting clinical signs of WNS should be released immediately, particularly if practitioners do not have the authority or confidence to euthanize a sick bat. If the bats will accept food and water, consider provisioning them to reduce any negative impacts on the hibernation energy budget (see "Provisioning bats").

Incidents of captures outside of hibernacula, and whether bats exhibit clinical signs of WNS, should be reported. Various groups, such as the permitting agency, the governmental agency responsible for the species, or other interested groups (e.g., CWHC), may be documenting and monitoring winter activity patterns and population health. Any dead or euthanized bats should be submitted for necropsy (see "Health Surveillance and Casualties").

Species. Species' responses to capture and holding vary. Northern Hoary and Eastern Red Bats, for example, hiss, produce clicking sounds, and jerk in the hand and holding bags

(Lollar 2018). These species, though, appear to benefit from being held in mesh bags, rather than cloth bags, and allowed to acclimate for 15–30 min before handling (CBWWG unpubl. data). Townsend's Big-eared Bat, although less reactive than lasiurines, also appears to experience acute stress when handled and to benefit from a 15–30 min acclimation period (CBWWG unpubl. data). Additionally, special requirements related to total holding time may be outlined in permits, particularly for species listed as endangered in the jurisdiction where a study is taking place. For more species-specific considerations refer to Table 2. However, individual variation in stress responses should also be considered when determining appropriate holding duration.

Collecting morphometric and demographic data

Although morphometric measurements (e.g., forearm and tragus lengths) are often useful for species identification, and demographic data (e.g., sex, reproductive status, and age) can be used to assess population health, obtaining each new piece of information prolongs handling and adds to the captured individual's stress. Hence, investigators must carefully identify which measures are required for project objectives and weigh these against increased handling time.

Morphometric and demographic data are also used when prioritizing species for release. Some of these data, like sex and often species, can be obtained quickly while removing bats from nets and traps. However, for other parameters like age and reproductive condition, bats must first be removed from nets and traps to collect the data. We, therefore, recommend assessing demographic status within 1 h of capture so that bats are not held longer than the suggested time frames (Table 3) and those needing more immediate attention, such as late-stage pregnant and nursing females or volant juveniles (see "Age class"), are processed in a timely manner.

Body mass. A bat's mass varies over 24 h (Hutson and Racey 2004), and individuals that are captured after peak foraging can weigh double what they do after evening emergence (CBWWG unpubl. data, Gould 1955). However, digestion times differ considerably within and across species (Roswag et al. 2012) and also vary with an individual's body temperature. Therefore, a fasted mass may be required to obtain an accurate measure of body mass. For example, a fasted mass may be needed in studies investigating seasonal fluctuations in body size (e.g., Reimer and Barclay 2024). Fasting is probably not necessary for most projects and is not crucial when bats are captured near the time of emergence, because they likely have not eaten since the previous night. Consider the necessity of obtaining a fasted mass against the potential risks associated with holding bats for the required duration (usually a 1 h minimum), as well as time to obtain other measures (refer to "Holding duration, General guidelines"). Factors such as the time of night and the state of the individual at time of capture (e.g., distended belly vs. emaciated) can inform how long to hold animals for obtaining a fasted mass.

Mass may be obtained using a spring scale (e.g., Pesola Micro, Chur, Switzerland) or a digital scale (e.g., AWS Pocket Scale, Cumming, GA). If using a spring scale, the clip can be attached to the bat bag. If using a digital scale, an extra bag or excess bag material can be wrapped around the animal to immobilize it, minimizing struggling and stress. Bats can also be temporarily restrained by wrapping them like a "burrito" in slightly stretched knee-high nylons, a piece of pantyhose, or similar stretchy fabric (Fig. 8). Animals can also be temporarily contained and weighed by placing them under a "mesh" desktop pen holder or paper cup. All materials and surfaces should be disinfected between weighing different individuals (see "Decontamination").

Forearm length. Forearm length is a standard measurement that indicates overall size of a bat and it is often used in conjunction with other morphological features to differentiate closely related congeners (e.g., *Myotis* species; Luszcz et al. 2016). Also, body mass is often divided by forearm length to determine a body condition index, although body condition index may be no better than body mass alone for estimating energy reserves for some species (CBWWG unpubl. data, McGuire et.al. 2018). Although measuring forearm length is a quick process, the handling time should be justified in advance for a given study. While a small plastic ruler may be used for less-precise measurements, workers typically measure forearm length using calipers, with one tip at the base of the bat's thumb and the other tip at the elbow, while avoiding potential damage to the skin, wing, and forearm (Québec Ministère des Forêts, de la Faune et des Parcs 2021, RISC 2022, Vonhof 2006). Sharp tips of metal calipers should not contact the bat, although this may occur if the animal is struggling; therefore, the tips may be dulled before use.

Additional morphometric measurements. The collection of additional morphometric data, such as the length of the tragus, ear, body, tail, and foot, may be essential in areas with species that are difficult to distinguish by visual inspection alone (RISC 2022, Vonhof 2006). Naughton et al. (2012) and species-specific literature, such as Lausen et al. (2022), should be consulted to determine which measurements and techniques are necessary to differentiate species in your region. Consider the additional handling time each measurement requires, and minimize holding time to comply with recommended safe thresholds (Table 3). Additional identification techniques (e.g., acoustic recording, genetics; RISC 2022) may also be needed for accurate species identification.

Sex. Males and females can be easily distinguished by gently examining external genitalia, to identify the penis or vulva (Racey 2009; Figs. 9–10). The maximum holding time may differ for males and females, due to different energetic demands that affect the

Figure 8. Bat wrapped in a nylon-fabric ("e.g., knee high stockings, also called pantyhose) "burrito" and placed on scale. Photo by Brock Fenton.

Figure 9. Comparison of female reproductive stages. Top left: nursing. Photo by Jared Hobbs. Top right: Late-stage pregnant. Photo by Jared Hobbs. Bottom: Post-lactating. Photo by Brock Fenton. See Racey (2009) for methods to assess reproductive stage.

amount of time needed to forage. Females may require shorter holding times than males (see “Reproductive status”).

Reproductive status. Maximum holding time may vary with reproductive status (e.g., pregnant and lactating), because foraging time to meet energetic demands and the costs of flight differ (see Table 3). To assess reproductive status of females (Figs. 9–10), consult Racey (2009). Generally, pregnant females can be identified by palpating the abdomen for a fetus, and nursing females can be identified by examining the nipple area for worn fur and by expressing milk (Figs. 9–10). Compared to females, there are fewer resources and standardized protocols for quantifying male reproductive status, but Lausen et al. (2022) is a useful resource.

Age class. Volant juveniles should be prioritized for processing, because they may not be proficient fliers (Buchler 1980) and may require more time to forage than adults. Brunet-Rossini and Wilkinson (2009) provide details for differentiating juveniles from adults, based on the presence of epiphyseal gaps in juveniles and “knobby” metacarpal joints in adults (Fig. 11). Tooth wear of the upper canines can also be used to identify juveniles and determine relative age among adults (e.g., Figs. 12–14; Supplemental File 1), although wear varies with species and regions due to differences in diet (Brunet-Rossinni and Wilkinson 2009, Christian 1956, Holroyd 1993). As always, consider the time needed to collect these data.

Torpor

Consider the implications of torpor for collecting data, as well as potential risks to animals’ welfare if holding and handling times are prolonged as a result of bats going torpid. For example, torpid bats are easier to handle and measure than normothermic individuals, but obtaining blood from torpid individuals is difficult (T. McBurney, CWHC Atlantic, PEI, Canada, pers. comm.). Also, bats recently aroused from torpor may not produce typical echolocation sounds (CBWWG unpubl. data), so recordings may not be representative of

Figure 10. Male bat. Photo by Jared Hobbs.

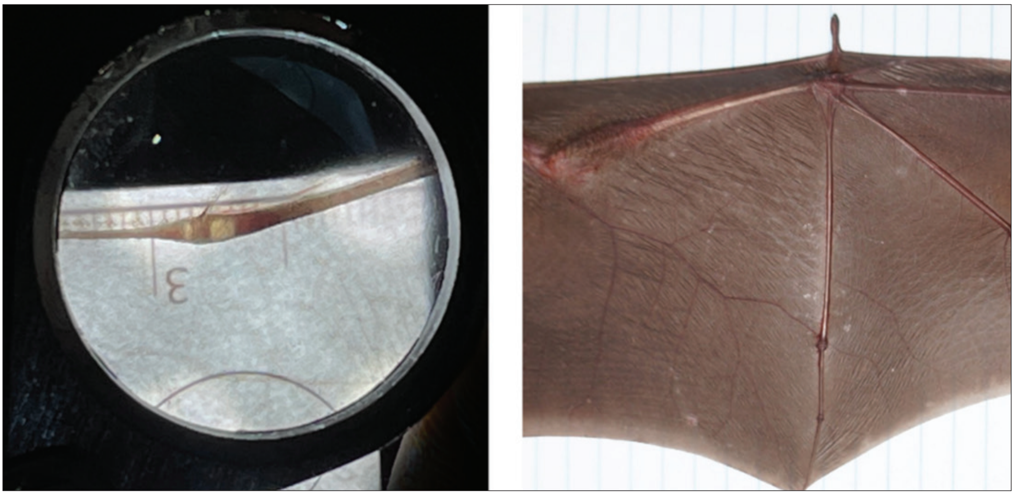

Figure 11. Left: Metacarpal-phalangeal joint of juvenile bat, showing epiphyseal gaps. Photo by Hildegard Gerhach. Right: Adult with "knobby" metacarpal joints. Photo by Krista Patriquin.

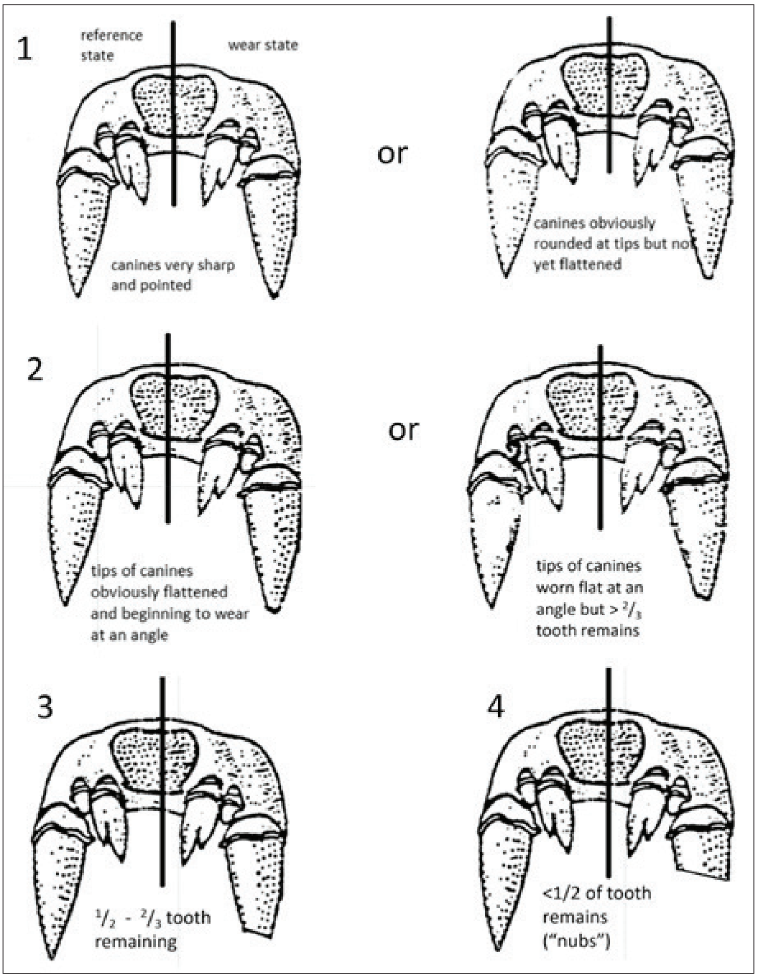

Figure 12. Schematic illustrating tooth class, based on tooth wear, that can be used for coarse-level age estimation (adapted from Holroyd 1993; original drawings from Christian 1956).

Figure 13. Top: Tooth class 1. Photo by Jason Headley. Middle: Tooth class 3. Photo by Krista Patriquin. Bottom Tooth class 4. Photo by Krista Patriquin.

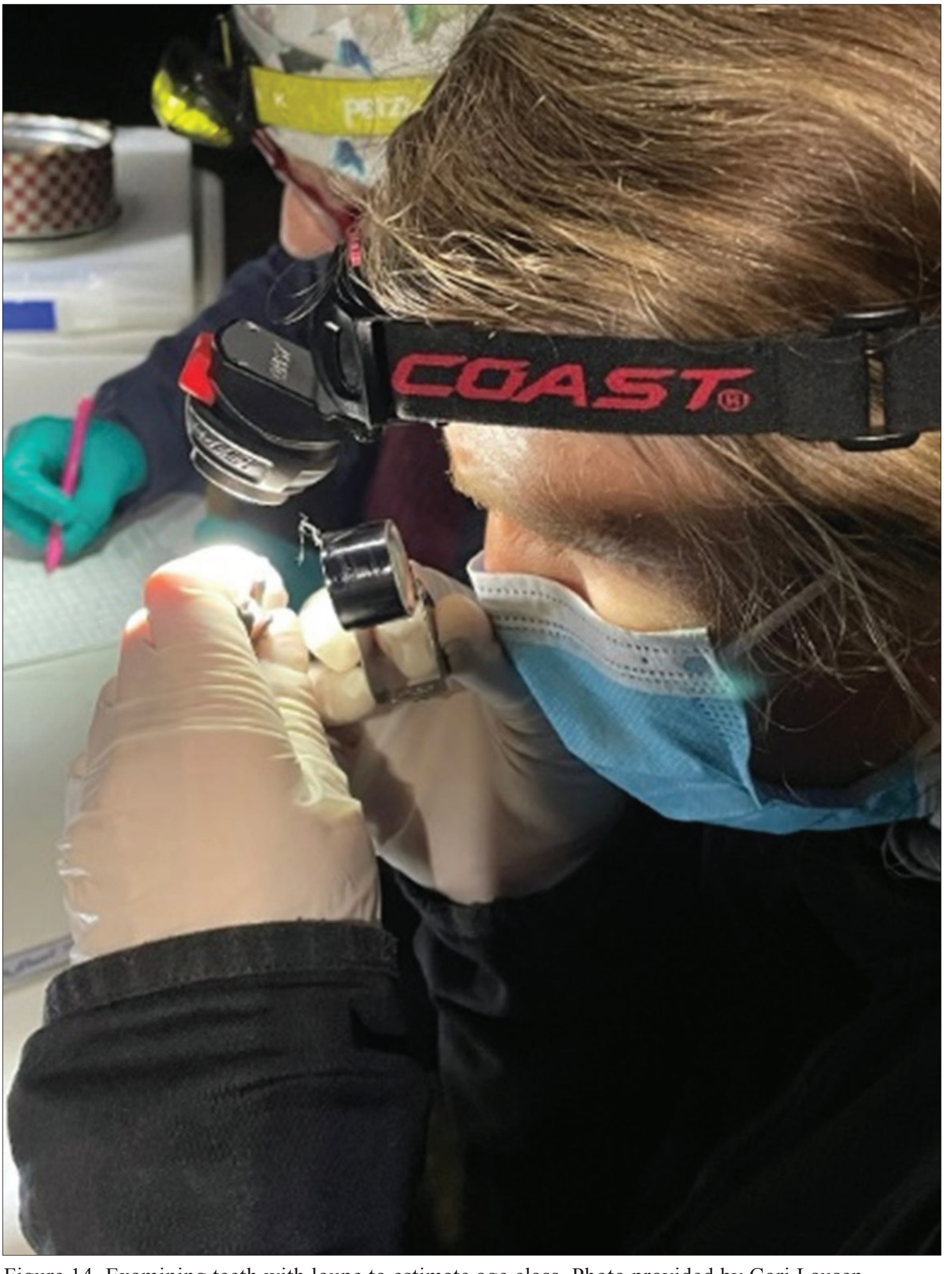

Figure 14. Examining teeth with loupe to estimate age class. Photo provided by Cori Lausen.

the species. Additionally, the energetic costs associated with remaining active, versus going torpid and rewarming before release, vary across demographic groups, which should be considered.

When not in flight, bats must expend energy to maintain their active body temperature (~37 °C) at ambient temperatures below their species-specific thermoneutral zones (Thomas et al. 1990). For instance, the thermoneutral zone for Big Brown Bats ranges from 26.7 to 36 °C; consequently, more energy is required at temperatures below this range (Willis et al. 2005). Although bats can opt to use torpor to save energy (Thomas et al. 1990), the frequency and depth of torpor varies with body size, reproductive stage, available energy reserves, and ambient conditions within and across seasons (Neubaum 2018). For example, small bats (e.g., Western Small-footed Myotis, California Myotis, and Small-footed Myotis) become torpid more often than large species (Stawski et al. 2014). Pregnant and nursing females often limit their use of torpor, because it inhibits fetal development and milk production (Besler and Broders 2019, Stawski et al. 2014). Bats may also limit use of torpor when captured on the landscape in winter or in other conditions that limit passive rewarming, because arousal is energetically expensive (Currie et al. 2015, Thomas et al. 1990). Therefore, we recommend investigators consider what is best for a bat regarding potential energetic costs of remaining active versus going torpid and being rewarmed before release. See "Holding duration – Season" for recommendations about holding bats and torpor use. If bats are allowed to go torpid, rewarm and provision them before release (see "Holding bags and bins" and "Provisioning bats").

Poor health

Free-living bats that have been captured and appear to be in poor health should be minimally handled, have no contact with other captured individuals, and either be released as soon as possible (see "Training") or, in some cases, examined by a wildlife health professional. The latter may be necessary if the unwell bat is unable to fly. Clinical signs of poor health include emaciation, dehydration, listlessness, and abnormal behavior. As always, be sure to wear gloves when handling bats, particularly when examining bats in poor health since the cause of their condition is unknown and may be an infectious disease transmissible to humans.

Emaciation is frequently diagnosed as a cause of death in Canadian bats (Beattie et al. 2022; Segers et al. 2021, 2024). Emaciation is evident when an individual's body mass is <40–50% of the species' reported maximum body mass, which is most often attained between late August and early fall. However, body mass varies widely with sex, age, and reproductive condition; moreover, mass fluctuates significantly with season, due to depletion or accumulation of fat related to changes in behavior (e.g., swarming in the fall) or physiological states (e.g., hibernation; Balzer et al. 2022, Gallant and Broders 2015, Jonasson and Guglielmo 2016, Jonasson and Willis 2011, Kunz et al. 1998, Lacki et al. 2015, Lausen et al. 2022, Naughton et al. 2012). Therefore, workers capturing and handling bats should be familiar with the factors that affect the body condition of the species they are studying to determine if a bat is emaciated. Clinical signs of extremely low body weight in a live bat include protruding shoulder blades and a concave abdomen, due to a complete absence of subcutaneous and internal adipose tissue stores, along with mild to moderate pectoral muscle atrophy (Beywig and Mitchell 2009, CWHC unpubl. data, Lollar 2018).

Bats may be dehydrated or become dehydrated following capture. The clinical signs of dehydration can include sunken eyes; stringy mucus; dry and tacky mucous membranes; dull, dry, and wrinkled flight membranes; and skin that stays tented when pinched (Lollar 2018).

Bats may also become listless following capture (CBWWG, unpubl. data). Listlessness is evident when the bat shows little or no reaction to stimuli, such as touch or light (i.e., does not open mouth, attempt to escape, or vocalize), and the body is limp when held, though the eyes may remain open. Listlessness may be confused with torpor. However, eyes are often closed while the bat is in torpor and the body is not limp when held. Also, overall responsiveness to stimuli increases in torpid bats as they warm, and they may also make audible vocalizations (CBWWG, unpubl. data).

If an emaciated, dehydrated, or listless bat is captured, researchers should use evidence-based knowledge and experience to assess the severity of the condition and the bat's potential for recovery, and to determine the best course of action. Depending on permit conditions and availability of veterinary care, this may include immediate release, provisioning with food and water, rehabilitation (if a facility is available), or euthanasia followed by necropsy to determine the cause of the emaciation, dehydration and/or listlessness.

Abnormal behavior can include extremely aggressive biting and struggling, uncoordinated movements, inability to fly, or acting very passive and unresponsive to stimuli. All these signs potentially indicate rabies (Constantine 2009). However, these behaviors also should be gauged in the context of the defensive actions associated with the stress of capture and handling, as well as what is considered "normal" for a species. For example, the passive, unresponsive clinical signs of rabies can be confused with listlessness and torpor. However, a listless bat should become more alert and responsive when it receives food, water, and rest, and a torpid individual should become more alert when warmed. Healthy Northern Hoary Bats and Eastern Red Bats hiss, click, and jerk when in the hand, and thrash in the holding bag, and normal Little Brown Myotis and Big Brown Bats often bite and produce loud, audible calls when handled (CBWWG unpubl. data, Lollar 2018). By contrast, some Pallid Bats are very docile when handled (Lollar 2018). Additionally, a bat may appear uncoordinated and be unable to fly if its body temperature is too low; for example, minimum body temperature needed for flight by a Little Brown Myotis is 30.3 °C (Studier and O'Farrell 1972).

To determine what is considered normal behavior, novices should consult literature and colleagues. Over time, experience leads to a better understanding of the range of normal responses that various species exhibit during capture and handling. A wildlife health professional (e.g., biologist that specializes in wildlife health) or veterinarian should examine a bat with abnormal clinical signs to determine if the animal should be submitted for post-mortem examination and rabies testing. If such an expert examination is not viable, place the affected bat in a safe site, where contact with humans or other animals cannot occur. If possible, monitor the bat for the next 24–72 h. If the bat dies or must be euthanized (see "Euthanasia") because clinical signs persist or worsen, submit the body for post-mortem examination (see "Health Surveillance and Casualties" and "Disposal of bats and waste materials"). Personnel should receive appropriate medical attention, if scratched or bitten by a suspected rabid bat (see "Vaccination").

Provisioning bats

Before provisioning bats, first ensure they are not rabid or otherwise sick (see "Poor health"). Always have water available for provisioning bats, especially if they are approaching the maximum holding time, if they are pregnant or nursing, or during sensitive seasonal periods, like winter. Use a sterile plastic eye dropper or syringe to deliver water to bats orally. To do this, allow a single drop to touch the bat's lips; normally, the animal responds immediately by licking the water. If a bat does not drink after being offered water twice, release the animal instead of prolonging its holding time (Lollar 2018). Similarly, if an individual appears severely dehydrated (see "Poor health"), release immediately.

It is also advisable to have food available to provision bats. The simplest and most nutritious option is canned cat food purchased from veterinary offices, because this type of food keeps for long periods, is easy to transport, and is typically higher in calories and contains more nutrients compared to cat food found in grocery stores (CBWWG unpubl. data). Soft cat food is preferred because it can be easily diluted with water and delivered with a plastic eye dropper or syringe. Other sources of energy to feed insectivorous bats while in the field include ferret food softened with water, and high-glucose veterinary supplements (e.g., Nutrical, Vetoquinol, Fort Worth, TX), though the latter contains fewer nutrients. Not all species or individuals willingly eat these items (CBWWG unpubl. data).

Mealworms (e.g., *Tenebrio molitor* Linnaeus [Yellow Mealworm]) are a common food for captive bats (Lollar 2018), and typically, only the wormlike larvae are used because the pupae and adults are often distasteful (Bat World Sanctuary 2014, CBWWG unpubl. data). Bats naïve to mealworms may not initially accept them as food. Therefore, removing the worm's head, and squeezing the viscera from the carapace onto the lips or into the bat's mouth may encourage ingestion (Lollar 2018). Once the bat licks the viscera, the individual often is willing to eat, and then whole worms can be offered. Mealworms differ substantially in size, and whole worms can be difficult for small bats (e.g., <5 g) to chew and swallow; therefore, they may only accept the viscera. To slow the growth of mealworms and delay metamorphosis, keep them refrigerated until needed in the field. A day or 2 prior to going in the field, consider "gutloading" mealworms to provide additional nutrients to bats. To do this, remove mealworms from refrigeration and provide them with nutrient-rich food sources, such as fruits and vegetables.

We advise feeding bats over a clean surface so that it is easier to retrieve dropped mealworms and prevent accidentally releasing them into the environment. To limit the risk of being bitten while feeding mealworms to bats, we recommend using forceps, but these should be non-metallic to prevent damage to the bat's teeth. To reduce the likelihood that bats bite the forceps, hold the mealworm in the tip of the forceps, with as much of the mealworm's body exposed as possible and as far from the tips as possible, then place the exposed portion of the mealworm near the bat's mouth. If squeezing the viscera into the mouth, you may have to use another pair of forceps. In this case, it is often easier to have 1 person hold the bat and another person present the mealworm to the bat. Decontaminate droppers, syringes, and forceps between use (see "Decontamination").

Releasing bats

Several factors should be considered when setting a bat free to ensure release is successful. Let the bat go in an open space with little vegetation, so it is easy to locate the animal if the release is not successful. The release should be near the site of capture during favorable weather (i.e., warm and dry with little wind). If a bat is not obviously ready to fly (i.e., not trying to escape or not flapping its wings), ensure that its body temperature is high enough to take flight. Perform a "test flight" by holding the animal near the base of the tail to encourage use of the wings (Fig. 15); a bat that is ready to fly will flap its wings quickly and powerfully. When ready, a bat can be released from your hand, while you stand, with arms extended above your head (i.e., ~2.5–3 m above the ground; Bowen 2020, Haarsma 2008). The height may need to be increased for large species, pregnant females and those carrying pups, volant juveniles, and individuals bearing radio transmitters; the effective height can be increased by standing on a stool, truck bed, log, or boulder. Do not release a bat over a stream from an elevated riverbank; if the animal cannot gain altitude after release, the bat may fall into the water and possibly drown (A. Kurta, Eastern Michigan University, Ypsilanti, MI, pers. comm.). When releasing a female with attached young, avoid placing a hand under the pups for security, because youngsters that contact the surface of your hand before release may unlatch from their mother (CBWWG unpubl. data). As much as possible, follow bats with a light to confirm they have flown away.

If a bat does not fly successfully, determine the cause and remedy it. Assess whether the animal is still torpid by repeating the test flight or allowing the animal to warm longer. In some cases, a bat may be warm enough but does not have sufficient clearance, so try releasing it again from a higher point. Bats may also not fly away successfully if the wing membranes are stuck together. Although the conditions resulting in sticky wings are not known, this adhesion can keep the wings from stretching out fully for flight. If a bat with sticky wings tries to escape or is released, it may drop to the ground when the wings do not fully open. To remedy this, gently unfold the forearm and index finger to open the wings manually and then perform a test flight.

If a healthy bat does not fly, despite repeated attempts, place the grounded animal high on a platform, ledge, tree trunk, or branch, in an area where the bat can crawl to a higher

Figure 15. Test flight. Photo by Krista Patriquin.

spot or shelter. Pick a location free of thick vegetation or tangled branches that may inhibit flight (Battersby 2010, Bowen 2020). This practice is not recommended near dawn because bats become visible to predators; therefore, plan to begin releasing bats well in advance of sunrise. As much as possible, monitor the animal to verify that it does fly away and, if it has not, reassess to determine if further action or intervention is warranted (see "Poor health").

Marking

In this section, we make general suggestions for using different types of marking (e.g., bands, PIT tagging, fur coloring, fur trimming, and radio transmitters) for short- or long-term identification and describe necessary precautions. Short-term identification involves recognition of an animal, such as a recaptured bat, within a night or season. Long-term marking is intended to provide an individual with a unique identification code for the animal's lifetime, often with the purpose of tracking changes to individuals and populations over time. A North American working group, which includes several CBWWG members, is currently reviewing best practices of common marking techniques and potential injuries (Cable et al. 2024, Loeb et al. 2025). However, recommendations were not yet available at the time of at the time of publication. We suggest interested readers visit the CWHC website (https://www.cwhc-rcsf.ca/bat_health.php), which is regularly updated with resources and best practices.

Short-term marking techniques

Water-soluble marker. Water-soluble, nontoxic markers can be used to color the hair of individuals to track recaptures within nights. Marks made with water-soluble markers may not be useful for tracking recaptures across nights, because grooming could remove the ink. Consider marking the animal's back because it is difficult to reach during grooming. Always read product labeling or specifications to determine if markers are nontoxic (e.g., Ultra-clean Washable Markers, Crayola, Easton, PA). Like other reusable equipment and materials, tips of markers should be disinfected before using on a different animal (see "Decontamination").

Nontoxic temporary coloring. Nontoxic temporary forms of coloring, such as paint, animal tattoo ink, and hair dyes without bleach, can be used to mark the hair of captured individuals to track recaptures within a season (see Lollar [2018], for recommended brands). Additionally, unique combinations of patterns, colors, and anatomical placements of dyes allow short-term identification of individuals. Dyes are not useful for marking individuals across seasons because bats undergo annual molts (Fraser et al. 2013). Always read product labeling or specifications to determine if paint and dyes are nontoxic and do not use bleach. Semi-permanent dyes used for pets (e.g., Dog Hair Dye, Opawz, Richmond Hill, ON, Canada) come in various colors and can be applied directly from the bottle to dry hair. To apply dye, it helps to place the individual in a restraining device (see "Restraining devices") to limit movement of the bat, to ensure dye does not contact the animal's eyes, and to allow the bat to rest until the dye dries.

Bee marking tags. Tiny colored and numbered discs normally used to mark bees (Supplemental File 2) have also been used to identify bats temporarily (Kirkpatrick et al. 2019). To attach tags, we recommend the use of latex surgical adhesive (e.g., Osto-Bond, Montreal Ostomy Center, Vaudreuil-Dorion, QC, Canada), but see "Adhesives" for a discussion of other types of glues. To apply the glue and tag, we advise placing the individual in a restraining device (see "Restraining devices") to limit the bat's movement, ensure glue does not contact the animal's eyes or wings, and to allow the bat to rest until the glue dries.

Hair removal. Hair removal may also be an effective method for temporarily marking individuals within a season. Hair is typically taken from the dorsum because ventral hairs are shorter and harder to access, increasing the possibility of accidentally cutting the skin during removal. Unless hair is being removed to attach radio tags, avoid the scapular region, where the skin overlies a prominent depot of brown adipose tissue; a lack of hair in this area increases heat loss when the animal is roosting. Instead, remove hair from the dorsal region near the tail. Do not remove hair near the wings to avoid accidentally lacerating them.

Use curved, blunt-tipped micro-dissecting scissors or cuticle scissors to remove hair close to the skin. Remove sufficient hair so that bare skin remains visible upon recapture; to check, brush remaining hair over the area to confirm that the cut patch remains evident (Fig. 16). Avoid removing excess hair, which could negatively influence thermoregulation. To minimize heat and water loss over winter, do not remove hair between early September and when bats return to warm-weather habitat in spring. Molting times vary across species, sex, and age, but new fur typically grows in the summer and fall (Fraser et al. 2013). Therefore, hairs removed late in the season may not be replaced until after hibernation.

Hair removed to attach radio tags or for biological samples (e.g., analysis of stable isotopes or heavy metals) can also be used to track recaptures at different locations or times

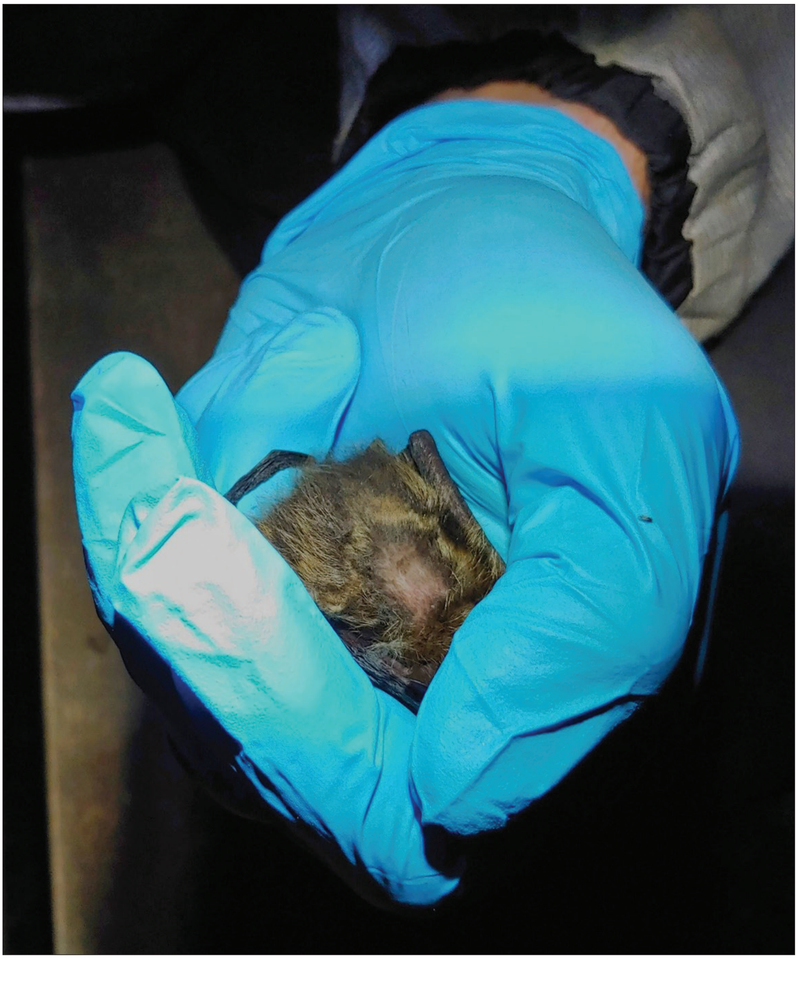

Figure 16. Hair patch removed for marking, sampling hair, and attaching radio-tag; patch would be smaller than shown if only for marking. Photo by Lori Phinney.

within a season. However, patterns of hair removal may not work for individual identification of bats when captured in large numbers, because Canadian species are small, which limits the number of unique combinations of patch removal.

Punch biopsies. If a project requires punch biopsies (see "Biological Samples"), the fresh or recently healed lesions from the punches can also be used to identify recaptures within a season. Typically, punch biopsies are acquired from wing or tail membranes, and healing can take 5–6 weeks (Ceballos-Vasquez et al. 2014); consequently, biopsies should be taken early enough during the warm season so that the wounds have time to heal before migration and hibernation. For details on safely obtaining punch biopsies, refer to "Punch biopsies" in "Biological Samples".

Light tags. Chemiluminescent (light) tags, commonly referred to as glow sticks, are useful for tracking an individual after release to obtain reference echolocation calls, or to observe flight and foraging behavior (Barclay and Bell 1988, Horvorka et al. 1996, RISC 2022). Those used with bats are small, nontoxic, miniature glow sticks that are 2.5-cm long or smaller (e.g., 1 Inch 24 Hour Mini Micro Glow Sticks, Glow Store, Victoria, BC, Canada). Tags can be attached directly to the fur with a nontoxic glue stick similar to that used by children in school (e.g., Disappearing Purple School Glue Stick, Elmer's Canada, Scarborough, ON, Canada; RISC 2022). Clipping hair is not necessary for attaching light tags. For small, low-flying bats (e.g., most species <20 g), tags should be placed on the back, as the abdomen may not be visible to observers. For large, high-flying species (e.g., most species >20 g), tags should be attached to the abdomen because the back may not be visible to land-based observers. Tags should not be placed on the abdomen of pregnant females near parturition or lactating females, because tags may interfere with nursing (RISC 2022). Carefully weigh the benefits of this technique against potentially increasing the risk of predation, which currently is unknown. One study revealed that light tags remain attached and glowing for up to 48 h (Timofieieva et al. 2019), but observations in the field also suggest bats readily land to groom these light tags off, especially if they are on the ventral surface (CBWWG unpubl. data, Horvorka et al. 1996).

Radio tags. Radio tags are used to track foraging patterns and habitat use, and for locating roosts. Locating roosts can provide insights on population health, because observers can conduct emergence counts to estimate colony size and track changes over time. Temperature-sensitive transmitters can be used to measure body surface temperature, which is used to examine thermoregulation and assess body condition at different reproductive stages (Belser and Broders 2019) and during hibernation (e.g., Jonasson and Willis 2011). Consult Supplemental File 1 and 2, respectively, for useful tips on successfully attaching a radio transmitter and for radio-tag manufacturers.

The smallest transmitter available should be applied to meet the research objective, while ensuring tags do not compromise flight. Generally, transmitter size is positively correlated with battery life, but this extended life comes with the cost of additional weight that could interfere with flight. Aldridge and Brigham (1988) calculated that the addition of 5% of a bat's body mass reduced maneuverability and required a 5% increase in power needed for flight. This led to the 5% "rule," which states the mass of a transmitter should be no more than 5% of a bat's fasted mass (Aldridge and Brigham 1988, O'Mara et al. 2014). This rule limited the ability to track small species, because transmitters of suitable weights were not available. However, tags have recently been developed that are light enough (e.g., 0.22–0.23 g) to attach to even small species, such as California Myotis and Small-footed Myotis (e.g., LB-2XT, Holohil Systems, Carp, ON, Canada; Moosman et al. 2023).

How broadly the 5% rule should be applied remains uncertain. For example, some studies have used transmitters comprising 5–10% of a bat's mass, with no apparent negative impact on movement (O'Mara et al. 2014). A bat's ability to carry a load, though, depends on more than body size; wing loading, or wing morphology relative to body size, also dictates ability to carry a load. Wing loading, in turn, varies by species, sex, age, and reproductive status (Meierhofer et al. 2024). Therefore, the 5% rule is a good principle to follow, but there may be some flexibility to this guideline.

Avoid placing radio tags on demographic groups with limited mobility, unless necessary to meet project goals (i.e., comparing thermoregulation across groups). Pregnant females, for example, have limited maneuverability because they are carrying a developing fetus, and the additional weight of a radio transmitter would further compromise flight. Similarly, the weight of a transmitter might hinder the flight of young juveniles that are not yet proficient fliers.

Adhesives. Several types of adhesives have been used for attaching tags to bats, including cyanoacrylate, surgical adhesives, and nontoxic glue sticks. Tags glued with cyanoacrylate (e.g., Super Glue, Super Glue Corporation, Ontario, CA) can remain attached for a full season between molts (Kirkpatrick et al. 2019). Although unpublished recapture data indicate no negative outcomes associated with the use of cyanoacrylate glue (OMNRF unpubl. data), we discourage its use. Unlike other adhesives, the safety of cyanoacrylate for use on bats has not been appropriately verified. If used, extreme caution is needed to ensure the glue does not contact a bat's eyes or wings, and the quick drying time makes it difficult to correct mistakes. In addition, inhalation of chemicals associated with cyanoacrylate adhesives may result in respiratory distress in small animals, and ingestion can cause gastrointestinal issues (Peterson 2016).

Tags glued with surgical adhesives can remain attached for days or weeks, depending on attachment method and environment. For instance, tags may stay on longer if glued to the skin instead of the fur. Although tags may not remain attached as long as those affixed with cyanoacrylate, the safety of surgical adhesive for use on bats has been verified (van Harten et al. 2020). We specifically recommend the use of latex-based surgical adhesives (e.g., Liquid Bonding Cement, Torbot Group, Warwick, RI), rather than those containing methacrylate, which may cause skin irritation or burns (Leggat et al. 2009). To minimize evaporation of volatile solvents and prolong effectiveness, store surgical adhesives in a refrigerator. Purchase new bottles each field season, because the glue thickens over time, which reduces effectiveness (Carter et al. 2009). Alternatively, thin older glue with an appropriate solvent, usually hexane, that can be purchased from the same manufacturer (but see product's Material Safety Data Sheet for other suggestions; Carter et al. 2009).

Nontoxic glue sticks, such as those used in children's classrooms and crafts (e.g., Disappearing Purple School Glue Stick), provide very short-term attachment of tags, because glue sticks are water soluble. Nontoxic glue sticks can be found in the stationary or crafting sections of most department and drug stores. Nontoxic glue sticks also pose the least risk of harm to bats, compared to the above-mentioned adhesives. It is, therefore, important to weigh the costs and benefits of different adhesives against project objectives.

Long-term marking techniques

Bat bands. There are no standardized banding protocols. Various band types (e.g., split-ring plastic or flat-lipped aluminum), sizes (e.g., inner diameters that provide a snug or loose fit), and application techniques (e.g., placed over the forearm or attached through incisions in the wing membrane) can result in damage if used incorrectly. Researchers in both the United States and Canada have increasingly used lipped metal bands (e.g., Porzana, Icklesham, East Sussex, United Kingdom) that come in several sizes and, when applied correctly, do not typically cut into the wing membrane. Additionally, banding pliers (e.g., Banding Pliers, Bat Conservation and Management, Carlisle, PA), customized to each band size, allow precise and repeatable application of ideal tightness (CBWWG unpubl. data).

As with all marking and sampling methods, banding should only be used if necessary and only by experienced individuals, with effective monitoring of bat health to limit negative impacts on banded individuals (Ellison 2008, Perea et al. 2024). For instance, in the US, Ellison (2008:69) suggests "that marking of bats with standard metal or plastic split-ring forearm bands not be considered for mark-recapture studies, or any study involving marked bats, unless the information sought and the potential for obtaining unbiased estimates from that information vastly outweighs the potential negative effects to the bats. Also, the inferences made from banded bats can never be extrapolated to the population level simply because banded and unbanded bats likely do not have the same fates, a major assumption of mark-recapture theory". The value of banding bats must, therefore, be carefully weighed against the potential risks to animal welfare.

A recent review of injuries resulting from banding in Europe led to a recommendation that banding as a marking technique be banned for all species (Lobato-Bailón et al. 2023). The suggested ban was based on a meta-analysis of literature reporting injuries to 8 species of free-ranging bats carrying lipped aluminum bands, compared to bats marked with passive integrated transponders (PIT tags). The review also included a controlled comparison of injuries resulting from lipped aluminum bands and PIT tags applied to members of a captive colony of *Carollia perspicillata* (Linnaeus) (Seba's Short-tailed Bat). However, the meta-analysis did not control for practitioner experience, and injuries varied by species. Therefore, while Lobato-Bailón et al. (2023) provide concerning data for the species they investigated, the authors' conclusions cannot be generalized. For example, a different study found that rates of injuries and mortalities among banded Little Brown Myotis were lower when snug (2.9-mm inner diameter) aluminum bands were applied, compared to loose-fitting (4.2-mm) aluminum bands and split-ring plastic bands (3.5-mm) (Reynolds et al. 2025). Additionally, injuries were temporary and individuals showed high rates of recovery (Reynolds et al. 2025). If choosing to use bands as a marking technique, carefully consider whether an alternative method can provide the same information or if the risk to individuals can be justified based on the 3 Rs (see "General Guidelines").

Some projects may warrant use of bands because they allow visual identification of previously captured bats without the need to recapture and handle the animals. Unique combinations of colors also allow quick identification of individuals, sex, age, capture location, etc. Additionally, bands may be a preferred marking option during the fall swarming season when implanting PIT tags is not recommended (see "Passive integrated transponders"). Bat practitioners should closely review future literature for recommendations about the best method for long-term marking. A good rule of thumb may be to employ a protocol similar to that in Australia—forego banding if it results in injury to >2% of recaptured bats (Baker et al. 2021).

If banding is necessary, biologists can choose from several commercial suppliers (Supplemental File 2). Generally, bands of any type can damage wings if not applied properly (Baker et al. 2001, Lollar and Schmidt-French 2002). Plastic split-ring bands designed for marking birds have been used for marking bats, but these bands have sharp edges where they split to open around the forearm. These sharp edges can damage or become embedded in wing membranes (Lollar and Schmidt-French 2002). To mitigate damage to wings, split-ring bands should be modified by trimming the edges with a nail clipper and using a nail file to remove any remaining sharp edges and widen the gap before applying the band (Fig.

17). Because bats can remove plastic bands or destroy identifying numbers by chewing the band, long-term projects should consider pairing these bands with PIT tags.

If individual identification from a distance is not needed, aluminum lipped bands specific to bats may be a good choice, because these bands have rounded edges that minimize wing damage (Fig. 18; Reynolds et al. 2025). Nevertheless, lipped bands are not without risk, if applied incorrectly (Baker et al. 2001). Retention of lipped bands for lasiurines is unknown and warrants investigation; it may be difficult to get a snug fit around the forearm because the propatagium is furred and larger compared to other insectivorous bats in Canada. Aluminum bands, like plastic bands, can be numbered or lettered for individual identification of recaptured bats, and anodized aluminum can also be colored (RISC 2022) to provide individual identification without capture. Lipped bands made from anodized incoloy (nickel-chromium alloy or magnesium-aluminum alloy) are also available (Supplemental File 2); these bands are harder and more durable than plastic or aluminum bands.

Always use the correct band size, and close the band an appropriate amount using finger pressure or banding pliers. The band should fit snugly enough to keep it from sliding over the wrist and to prevent finger bones from getting caught when the wing is folded. However, the band should not be so tight that it hinders grooming of the membrane underneath or pierces the wing membrane. The band also should fit loosely enough so that it moves freely up and down the forearm without causing abrasion or tears. Some practitioners choose bands with an internal diameter equivalent to ~7% of a bat's forearm length, with the smallest band height and largest gauge possible (CBWWG unpubl. data). Evidence to support these recommendations comes from a comparison of injuries and mortalities to Little Brown Myotis, resulting from split-ring plastic bands and lipped aluminum bands of different sizes (Reynolds et al. 2025). Injury rates were lower when appropriately sized (2.9-mm inner

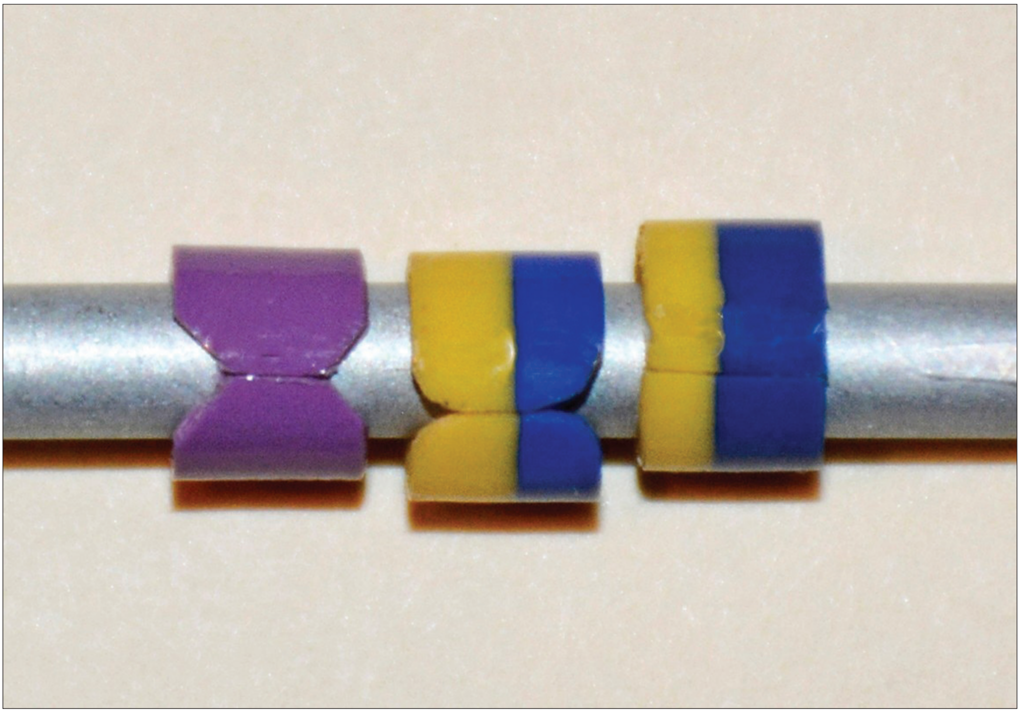

Figure 17. Plastic split-ring bands that have been modified for use (left and center), compared to an unmodified band (right). Photo by Robert Barclay.

diameter) aluminum bands were applied, compared to larger (4.2-mm) aluminum bands and split-ring plastic bands (3.5-mm; Reynolds et al. 2025). Make sure several band sizes are available to accommodate inter- and intraspecific variation in forearm size.

Regardless of type, check band integrity before use in the field. Discard bands with edges that do not align properly when closed, which could result in small lacerations of wing membranes (OMNRF unpubl. data). Banding pliers are helpful for applying metal bands; however, pliers should be tested and labeled before use in the field, to confirm they match the band size and result in proper closure on the forearm. Narrow 90°-angled circlip pliers (or similar) are useful for removing bands if they are applied too tightly/loosely or if bands are to be removed from recaptures at the end of a study. If band application or removal results in a bat bleeding, it can be stopped by applying direct pressure or a hemostatic agent, such as silver nitrate sticks (e.g., AMG, Medpro, BC, Canada) or coagulating powder (e.g., Blood Stop, Dominion Veterinary Laboratories, MB, Canada; Kunz and Weise 2009).

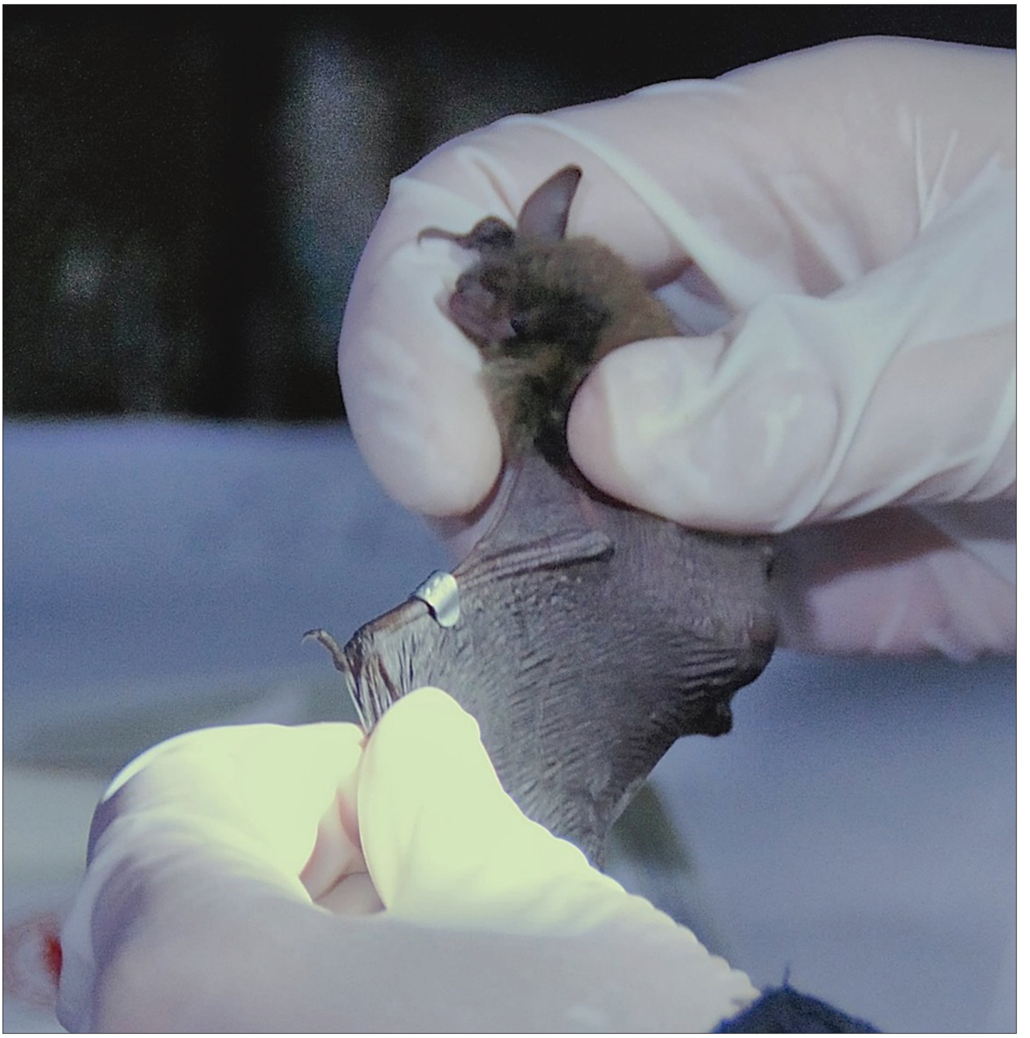

Figure 18. Lipped aluminum band. Photo by Brock Fenton.

Sikes et al. (2016) suggest inserting a band through a small incision made in the plagiopatagium of the wing membrane immediately adjacent to the forearm. Presumably, this practice is intended to limit the movement of bands along the forearm and minimize potential injury to the wing membrane. However, this procedure is generally discouraged for most Canadian species because it is invasive and unnecessary. This technique may be useful when applying plastic split rings or metal bands (without lips) on Northern Hoary Bats and Eastern Red Bats because their propatagia are larger compared to other Canadian species. Bands may, therefore, rub and get caught on their propatagia. However, long-term outcomes of such incisions are not documented, and they should be performed only by the most experienced practitioners, preferably with subsequent monitoring to assess the safety of the technique.

Basic guidelines for applying bands are provided in Supplemental File 1. A video demonstrating application and removal of bat bands can be found at https://www.youtube.com/watch?v=2_jiAzNFde0. Inexperienced personnel should practice placing and removing bands on a bat carcass, if available; otherwise, use a suitable-sized twig or wooden dowel.

Passive integrated transponders. Radio frequency identification (RFID) tags, such as passive integrated transponder (PIT) tags and microchips, are commonly used to mark small mammals, including bats (Jung et al. 2020, Waag et al. 2025). PIT tags are injected subcutaneously (i.e., under the skin but not in the muscle tissue) and have unique alphanumeric codes that can be detected and recorded with portable handheld readers or permanent readers installed at openings of roosts. Injecting PIT tags, though, requires training and increases handling time, as well as project costs, so we discourage use of PIT tags for studies that require only short-term identification. Instead, PIT tags are best suited for long-term population monitoring and behavioral studies that involve passive monitoring of marked individuals. Nevertheless, the application of PIT tags is discouraged in late fall and winter because associated wounds may not heal during hibernation (Ceballos-Vasquez et al. 2015). Tags come in different sizes (lengths), and those that are 12-mm long appear to be more reliable for long-term detection compared to 9-mm tags (Sandilands and Morningstar 2021). However, smaller tags may prove equally useful (OMNRF, pers. comm.), particularly for studies of small species, such as the Small-footed Myotis, because 12-mm tags may be too large.

For projects requiring PIT tags, train personnel to perform subcutaneous injections. This could include practice on objects like a chicken breast with skin still attached or a bat carcass when available. One person may restrain the bat, while another person inserts the tag. To prevent inserting the needle into the muscle tissue, the person injecting the tag should use their fingers on the non-dominant hand to apply gentle pressure at the injection site on the bat to feel for the needle tip. While continuing to apply gentle pressure on the animal, push the syringe plunger with the dominant hand to eject the tag, and use the fingers on the non-dominant hand to feel the tag as it is inserted under the skin. An apparatus like the McMaster restraining device (Ceballos-Vasquez et al. 2014; see “Restraining devices”) can limit a bat’s movement and reduce risk of injury. Consult Supplemental File 1 for instructions and tips to inject a PIT tag safely. Interested readers can also view a video here: https://www.youtube.com/watch?v=pVxoEL4YJKE .

Marking methods that are not recommended

Several marking methods are no longer recommended, including tattooing, freeze marking, ear punching, fur bleaching, nail and toe clipping, and use of beaded necklaces (CCAC 2003, Kunz and Weise 2009, Sikes et al. 2016). Specifically, freeze marking and fur bleaching result in tissue damage if done improperly (Silvy et al. 2005). Clipping nails, toes, or ears is markedly invasive and can interfere with grooming, roosting, navigation, or foraging (Kunz and Weise 2009), whereas tattooing requires extensive training and can be time consuming (but see Markotter et al. 2023). Bead necklaces may result in choking, abrasion, skin irritation, and increased predation (Jackson 2003, Kunz and Weise 2009). Consequently, these methods are strongly discouraged.

Biological Samples

Before collecting biological samples, investigators should apply for the required permits to obtain the samples and to ship materials to the appropriate laboratories for analysis. Although tissues and fluids, like milk, blood, and urine, can provide valuable insight on health and movement (e.g., migration; Brewer et al. 2021), they are not required for routine surveys and can require longer handling times compared to hair or punch biopsies. Consult the following sources if interested in obtaining these samples: tissues (Brewer et al. 2021), milk (Kunz and Parsons 2009), blood (Eshar and Weinberg 2010, Hoffmann et al. 2010, Hooper and Amelon 2014, Kunz and Parsons 2009, Smith et al. 2010), and urine (Bassett 2004, Greville et al. 2022, Kunz and Parsons 2009, Pilosof and Herrera 2010). However, a bat caught in a mist net often urinates when first touched, and workers can collect the urine by holding a capillary tube over the genitals before extracting the animal from the net. This technique, however, is not recommended for the Northern Hoary Bat and Townsend's Big-eared Bat because they appear to display acute stress when initially captured and may benefit from an acclimation period (~30 min) before handling for sample collection (CBWWG unpubl. data; Table 3).

Punch Biopsies

Biopsies from the wing or tail membranes are used for genetic analyses (e.g., species identification or relatedness), and from the muzzle and wing membrane for the diagnosis of WNS (Meteyer et al. 2009). Similar to other procedures, personnel should receive appropriate training before obtaining biopsies from live bats, including practice on a fresh bat carcass or on a non-animal model, such as a lightly stretched surgical glove. The McMaster restraining device (see "Restraining devices") limits animal mobility and, therefore, mitigates against potential harm during this procedure.

Ease of obtaining biopsies, and their quality, depend on the membrane from which samples are obtained. Wing membranes are easier to access, and portions of them are less vascularized than the uropatagium, thus reducing the risk of excessive bleeding (Hoffmann et al. 2010). However, the tail membrane heals faster, and samples from this location contain a higher concentration of DNA than those from the wing (Faure et al. 2009). Nevertheless, Broders et al. (2013) reported a Northern Myotis accidentally caught on a car antenna through a biopsy hole in the uropatagium, as well as a record of a torn tail membrane at the biopsy site. The potential for damage to the tail membrane after release may be greatest for species that frequently rely on the tail membrane for capturing prey, especially those that glean. For these bats, consider taking samples from wing membranes instead. However, also consider location on the wing membrane when obtaining biopsies, because vascularization and air flow differ dramatically across the wing's surface (see Supplemental File 1 for suggested wing area to biopsy; Figs. 19–20). The time to heal from the resulting holes may vary with species, season, and energetic demand, but wounds generally heal in 2 weeks (Weaver et al. 2009).

To obtain a sample, stretch the wing or tail membrane (Fig. 19) over a firm surface (e.g., cutting board or surface of the McMaster restraining device; Fig. 21). A 2-mm biopsy punch is appropriate for the Canyon Bat and Tricolored Bat, whereas a 3- or 4-mm punch may be used on larger species (RISC 2022). As always, disinfect materials between use (see "De-

Figure 19. Left: Wing extended for biopsy. Photo by Donald Solick. Right: Tail stretched for biopsy. Photo by Jordi Segers.

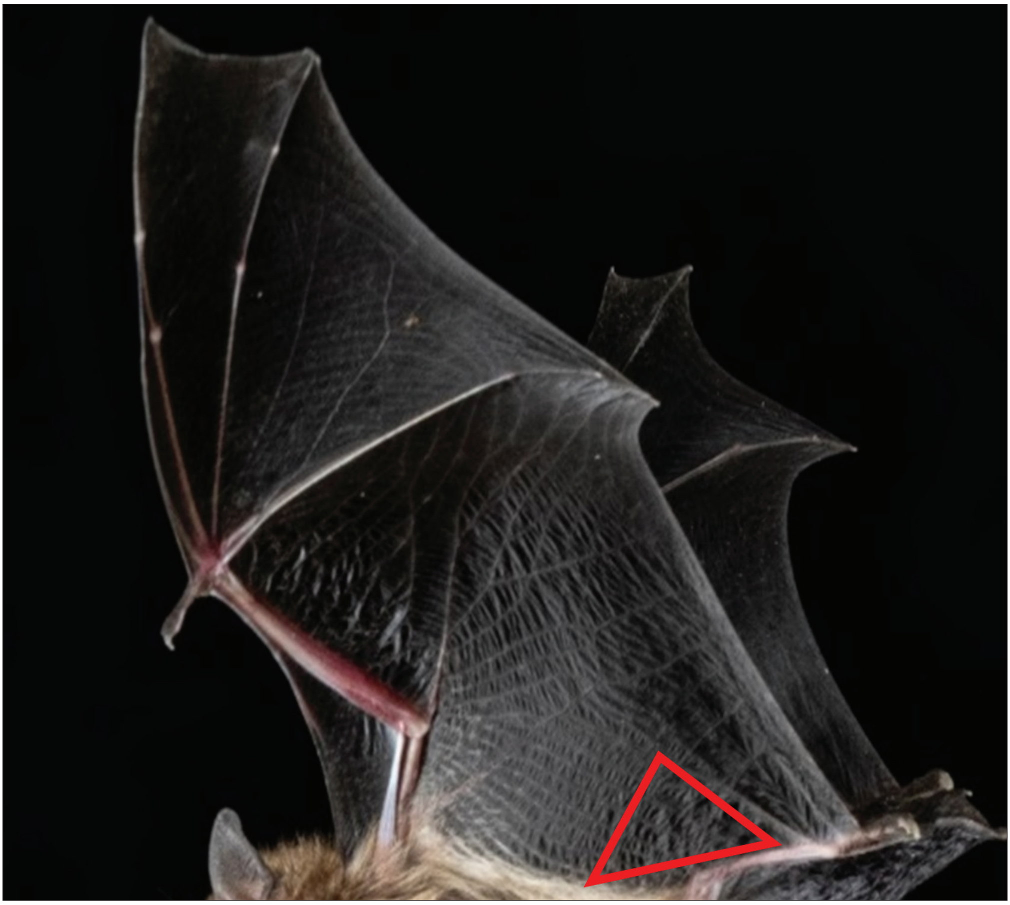

Figure 20. The "magic triangle" (outlined in red) for biopsies. Adapted from photo by Brock Fenton.

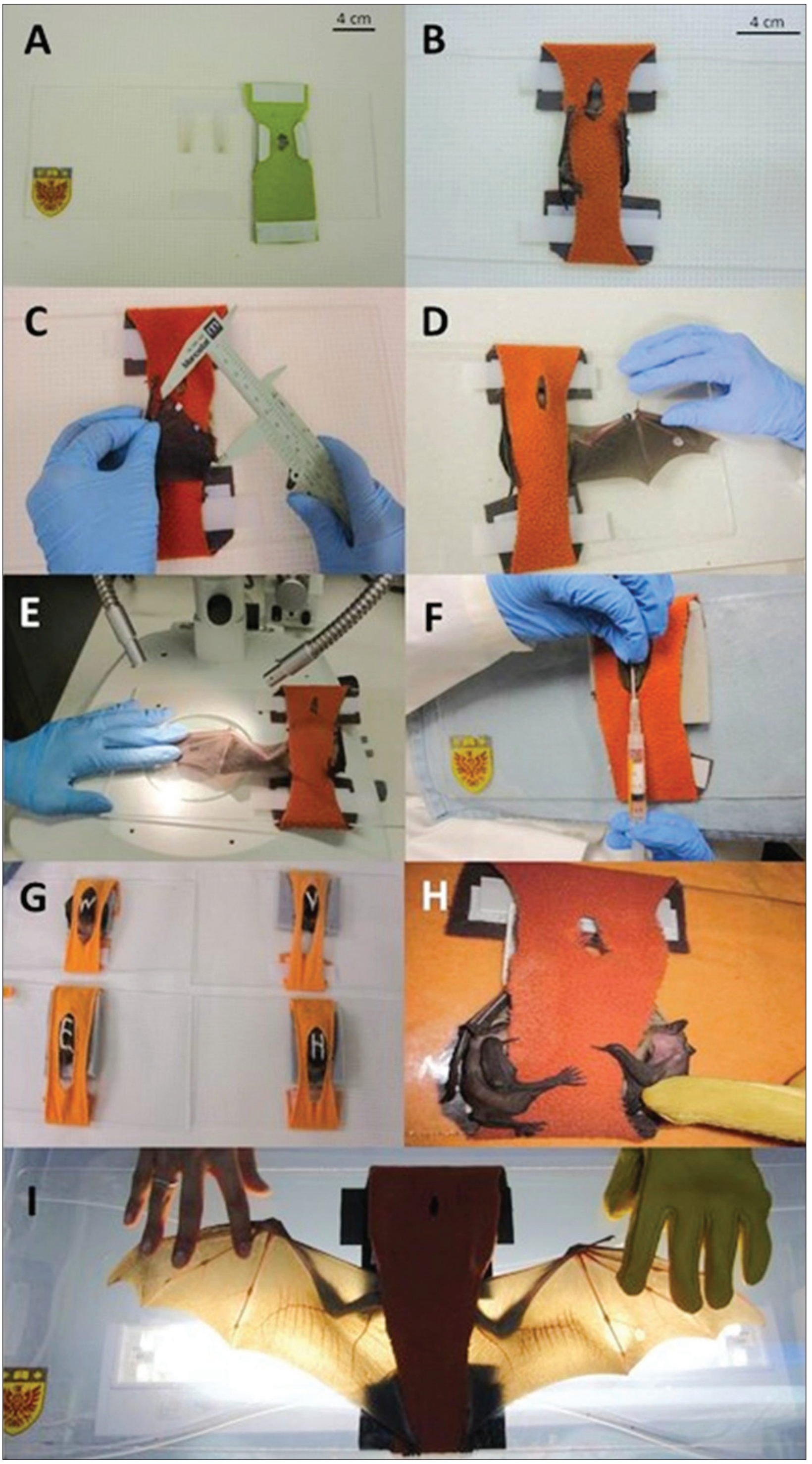

Figure 21. Bat in McMaster restraining device with transmitter. Image courtesy of Paul Faure, from Ceballos-Vasquez et al. (2014).

contamination"). Detailed instructions for obtaining biopsies appear in Vonhof (2006), and updated procedures are provided in Supplemental File 1.

Hair

Hair may be used for isotopic analysis to examine migratory movements and diet (Brewer et al. 2021, Campbell et al. 2017). The isotopic signatures may differ depending on the anatomical region of hair removal, and up to 1 cm^3 of hair may be needed (Brewer et al. 2021, RISC 2022). Therefore, carefully consider the potential impacts of hair removal on thermoregulation. For guidance on hair removal, see "Hair removal" in "Short-term marking techniques".

Fecal samples

Fecal samples are used for varied purposes, including studies of diet, hormones, and endoparasites (Kunz and Parsons 2009). Feces are often easily obtained from within the holding bags of bats that have been kept for 30–60 min, when capture occurs at least 1 h after emergence. Storage of samples depends on the project's objectives. For example, feces may be collected in a small vial with silica gel as a desiccant that helps preserve DNA for later analysis (USFWS 2024), but silica gel is not essential for visual dietary analysis (e.g., Painter et al. 2009).

Ectoparasites

Some studies require examination of ectoparasites (Czenze 2011, Poissant 2008, Whitaker et al. 2009), although more commonly, practitioners opportunistically collect information about simple presence of ectoparasitic species (e.g., Sauk and Broders 2025). If collecting parasites, the best form of removal depends on the parasite. For example, wing mites (Spinturnicidae) and ear mites (Trombiculidae) can be collected by passing a cotton swab dipped in ethanol over the wing or ear, which may also work with fleas (Ischnopsyllidae) (CBWWG unpubl. data). Bat bugs (Cimicidae) and mites holding on to the bat's skin or hair can be removed using forceps. The use of flexible forceps with a flat head helps prevent crushing the ectoparasites and allows mites holding onto the bat's skin or hair to be pulled gently or scraped out, as well as fleas to be grabbed as they move quickly through the fur. Before SARS-CoV-2, blowing on the fur assisted in moving hair out of the way for observation of the underlying skin. However, due to concerns about pathogen transmission from humans to bats, blowing on animals has been replaced by alternative techniques (see "Mist nets"). Any parasites that are collected can be stored in vials of 70% ethanol for later identification in the laboratory.

If close examination is required, use a dissecting scope and a tool, such as forceps, to part the hairs for systematic examination of the bat's dorsal and ventral surfaces. Be sure to inspect nostrils, internal surfaces of the ears, and urinary and genital openings, as well as hairs and membranes (Whitaker et al. 2009). Although more parasites are found with such a detailed inspection, additional equipment, like a scope, may be impractical in the field, and remember that close examination requires extended handling times.

Photography

Taking photographs is warranted for various reasons, including education, training (e.g., demonstrating handling and marking techniques on bats), species verification, obtaining data on wing morphology (e.g., size and shape), and documenting wounds, wing scarring, traumatic injuries, or other unique features. Nevertheless, photography increases handling and holding times, and consequently, "trophy" and social media pictures of bats are generally discouraged, unless they are to be used for outreach. Tips for taking photographs of bats while minimizing stress on the animals are available from the White-nose Syndrome Communications and Outreach Group (2023).

Euthanasia

Euthanasia is derived from the Greek terms meaning "good" and "death" and, in the context of this monograph, it means purposefully ending the life of an individual in a way that minimizes or eliminates pain or distress (American Veterinary Medical Association [AVMA] 2020). Investigators participating in field activities should plan for the possibility of euthanasia during their work. This preparation includes considering the endpoints, or decision rules, indicating when euthanizing captured bats is appropriate. Potential endpoints may include the occurrence of unanticipated life-threatening injuries caused by capture, or the identification of significant health problems during handling that would likely result in mortality of affected individuals. Other considerations before initiating a project include having the proper permits for euthanasia, receiving suitable training in the necessary techniques (see "Training"), and obtaining the needed materials to accomplish the task. In addition, workers should recognize that any procedure for euthanizing animals must also prevent or minimize risk to the safety of humans and the environment (AVMA 2020). Therefore, investigators should develop standard operating protocols for the best techniques of euthanasia, ensuring a high standard of care for bats, adherence to good principles of animal welfare, and appropriate biosafety measures. Routinely verify protocols are current for personnel training by re-evaluating them on a regular basis (i.e., every 2–3 years), documenting the most up-to-date techniques, and adopting the most recent technologies and animal welfare practices.

Though rare, circumstances arise during fieldwork when bats should be euthanized. For example, euthanasia may be warranted for animals that sustain life-threatening injuries during capture or handling. Severe injuries include, but are not limited to, fractures of the skull or long bones (e.g., humerus or femur), long tears in the skin of the body that cannot be immediately repaired, deep lacerations with exposure of underlying organs, tears in the wing membranes that preclude normal flight, and significant bleeding. Assessment of blood loss in bats can be difficult. In mammals, loss of >15% of total blood volume can lead to hypovolemic shock, a life-threatening condition in which severe blood loss prevents the heart from pumping enough blood to the body's tissues, leading to organ failure and death; in Little Brown Myotis, which has a total blood volume of ~1 ml, a loss of only 150 µl of blood may require medical intervention or euthanasia (Hall and Drobatz 2021, Hooper and Amelon 2014). If moribund or sick bats are found on the ground or are unable to fly, capture and submit them to a wildlife veterinarian or a licensed rehabilitator for examination. If a veterinarian or rehabilitator is not available, euthanasia may be appropriate.

Successful euthanasia requires rapid unconsciousness, followed by immediate cardiac or respiratory arrest and, ultimately, loss of brain function (AVMA 2020, Lollar 2018). Although achieving both goals is possible with a single agent, most techniques used for such euthanasia involve injectable drugs, which require adequate blood perfusion for the drug's effectiveness. If a bat is in hypovolemic shock, adequate blood perfusion is not likely, resulting in prolonged pain and stress. Thus, euthanasia is frequently a 2-step process, the first involving an agent to depress or eliminate functioning of the central nervous system, followed by a second step to stop the heart (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). The first action renders the animal unconscious and insensitive to pain, while the second step causes death.

The currently preferred method to achieve euthanasia of small insectivorous bats (i.e., ≤30 g) also requires two steps, including first to use an overdose of inhalant anesthetic to ensure rapid unconsciousness, followed by manual cervical dislocation as the second step to ensure death. However, the CCAC (2003) only recommends an overdose of anesthetic gas for use in bats ≥30 g, believing their size precludes the effective use of cervical dislocation. Since the CCAC (2003) publication, data from domesticated species used in research suggest manual cervical dislocation, without the use of tools, may be appropriate for rodents <200 g (AVMA 2020). The largest Canadian bat, the Northern Hoary Bat, weighs well below 200 g; maximum weight is 37.5 g, and average mass is 30.0 ± 0.6 (*SE*) g for adult females and 23.4 ± 0.6 g for adult males (Koehler and Barclay 2000, Naughton et al. 2012). Therefore, the effective use of cervical dislocation may be possible for bats ≥30 g. As a result, we recommended the double method (i.e., an overdose of anesthetic gas followed by manual cervical dislocation) for euthanasia of all Canadian species. However, flexibility is required in fieldwork, and some unforeseen events might result in manual cervical dislocation alone as the only viable option.

An overdose of inhalant anesthetic usually involves placing the compound isoflurane in an airtight chamber (see "Open-drop method") to cause rapid unconsciousness and a generalized depression of the bat's central nervous system, eventually leading to cessation of breathing and death (AVMA 2020). Sevoflurane is a similar gas, but it is not recommended for the open-drop method because concentration of this gas cannot be accurately controlled with this procedure (Institutional Animal Care and Use Committee of the University of Iowa [IACUC] 2023). Isoflurane is not a controlled substance in Canada, but a prescription is required for its purchase and can be obtained through a veterinarian. Additionally, to protect their health, personnel using isoflurane should consult its current Material Safety Data Sheet (MSDS). Isoflurane must be used only in a well-ventilated outside environment or in a fume hood to prevent accidental exposure of personnel, especially pregnant individuals due to the potential risks to a developing fetus. A bottle containing isoflurane should never be transported in enclosed spaces, such as the cockpit or cabin of an aircraft, or passenger compartment of a vehicle. Instead, the bottle of isoflurane should be placed in an outside compartment (i.e., trunk, baggage compartment, or truck bed), within a crush-proof and leak-proof container, preferably encased in additional shock-absorbing material to prevent breakage of the bottle. Additionally, never take isoflurane into enclosed spaces, such as hibernacula or summer roosts.

Open-drop method

The open-drop method (also known as "drop jar method") is an anesthetic procedure involving the use of a volatile anesthetic, like isoflurane, in an open manner by placing it directly in a small, covered chamber and allowing the volatile anesthetic to vaporize and be inhaled by an animal placed inside the chamber. This contrasts with the medical delivery of a volatile anesthetic via a closed, precision vaporizer through a nose cone or endotracheal tube. The materials and equipment required for the open-drop method are minimal and include isoflurane, disposable gloves, protective eyeglasses, an airtight chamber, cotton balls, a 5-ml syringe, and a perforated metal container (e.g., a ball strainer for tea). For Canadian species, a chamber with a volume of 250–500 ml is adequate to hold an individual comfortably. The chamber can be plastic or glass, but should be transparent to allow observation of the animal to assess for signs of distress. Isoflurane should be tested in the chamber before euthanasia, because some plastics (e.g., hard, clear, polystyrene plastic) can chemically dissolve on exposure to isoflurane and subsequently entrap the enclosed bat in the sticky residue (CBWWG unpubl. data). If a glass chamber is chosen, it must be protected against breakage during transport. Mason (canning) jars of appropriate size are well suited for this technique (CBWWG unpubl. data); if the rubber seal on the snap lid degrades (i.e., becomes sticky), it can be easily replaced.

The concentration of isoflurane required to euthanize a bat has not been reported, but a concentration of 5% isoflurane or greater must be reached in a container to euthanize a bird of any size (IACUC 2023). IACUC (2023) reports that 0.25 ml of isoflurane in a 1000-ml container produces a 5% concentration (i.e., 0.0625 ml isoflurane/250-ml container or 0.125 ml isoflurane/500-ml container). However, if in doubt about the volume of isoflurane to use, err on the side of a larger amount to induce certain, rapid, and controlled effects (CCAC 2010). Therefore, we recommend using 1–2 ml of isoflurane for the open-drop method, which takes into consideration the following: there is currently a dearth of literature to suggest an appropriate concentration of isoflurane for euthanasia of a bat; bats can enter torpor causing them to take less frequent and more shallow breaths compared to active bats (see below); field conditions can involve working in temperatures below 0 ºC, which affects the vaporization of isoflurane (see below); and certain, rapid, and controlled euthanasia of the bat are the goals. Therefore, after donning disposable gloves and protective eyeglasses in a well-ventilated area, use a syringe to draw 1–2 ml of isoflurane from its bottle and apply a sufficient quantity to saturate a cotton ball that is held in an open tea strainer. Be sure that there is no free-standing or dripping liquid, and close the strainer before putting it in the euthanasia chamber, to prevent contact of the bat with liquid isoflurane (AVMA 2020, IACUC 2023), which is a skin and eye irritant (review product's current MSDS). Once placed in the chamber, the bat should be monitored regularly for any signs of distress, such as struggling, excessive grooming, or self-mutilation. The closed chamber can be covered with a cloth or towel between observations to minimize stress.

Allow at least 15 min for unconsciousness and cessation of breathing to occur. If these have not been achieved, the bat can be left in the chamber for a longer period. However, cessation of breathing is not a sufficient criterion of death, and proper technique includes a follow-up examination to confirm death (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). Standard evidence of death includes lack of withdrawal and palpebral reflexes (i.e., failure of a limb to pull back from a toe pinch or touch of the eye to cause eyelid movement, respectively), as well as loss of muscle tone, resulting in relaxation of wings and legs when extended. Even if all of these criteria are met and death is apparent, manual cervical dislocation is also recommended (see "Manual cervical dislocation") to ensure the animal's death.

The biology and ecology of bats raise additional considerations regarding use of the open-drop method. Bats that are in torpor, either during hibernation or in cold ambient temperatures (usually <10 °C), have markedly reduced respiration, so cessation of breathing is difficult to assess. If possible, transport the bat to a well-ventilated, warm area (i.e., >20 ºC) to bring the animal out of torpor before euthanasia. However, if the bat cannot be brought to such an environment in a timely, stress-free manner, manual cervical dislocation may be performed as the sole method of euthanasia. Furthermore, cold temperatures in winter and throughout the night at Canadian latitudes, even in summer, may not allow isoflurane to vaporize sufficiently to achieve the recommended 5% or greater concentration required for the purpose of euthanasia. Schenning et al. (2017) demonstrated that a temperature of approximately -13 °C or warmer is required to deliver a 5% concentration of isoflurane in a closed system, using a digitally controlled thermoelectric anesthetic vaporizer. Therefore, manual cervical dislocation is recommended as the sole method of euthanasia and the most humane approach at temperatures below -13 °C.

Manual cervical dislocation

Although manual cervical dislocation is preferably performed as the second technique in the 2-step process of euthanasia, this method can be used as the primary technique by trained individuals in certain circumstances, such as when the open-drop method cannot be effectively administered (see “Open-drop method”). Manual cervical dislocation separates the brain from the spinal cord and tears the blood vessels supplying the brain, resulting in rapid death. Manual cervical dislocation requires no special equipment other than gloves to protect a person’s hands from being bitten or scratched during the procedure. However, it requires skill, training, and physical hand strength, and, thus, new practitioners should first practice on dead specimens to confirm they can perform the technique and become proficient, to reduce distress in the bat to be euthanized (AVMA 2020). Ideally the carcasses would be from bats that had recently died or had been euthanized by a trained individual. However, carcasses for practicing cervical dislocation might be more readily available from other sources, including museum specimens and diagnostic laboratory specimens that have tested negative for rabies virus. The use of a freshly dead bat avoids the influence of rigor mortis and post-mortem decomposition, both of which can markedly alter the normal feeling of the cervical anatomy. Thus, practice with types of specimens other than freshly dead bats may make it less likely that inexperienced individuals can competently perform the technique during an actual euthanasia.

With gloved hands, hold the bat horizontally so that its abdomen, chest, and chin are resting on a flat, firm surface (e.g., clipboard, tote box lid, wooden board, or flat rock). Place the thumb and middle finger of your dominant hand laterally on both sides of the base of the bat’s skull. Using the index finger of the same hand, apply a firm downward pressure on the dorsal surface of the first cervical vertebra, where it attaches to the base of the skull. With the opposite hand, grasp the base of the tail and quickly pull backward so that dorsal pressure from the other hand’s index finger separates the first cervical vertebra from the base of the skull. A pop may be heard or felt as separation occurs, and cervical dislocation can be confirmed by palpation of the neck. As indicated above, observe the animal for lack of responsiveness and cessation of breathing, and confirm death with an appropriate follow-up examination. For those that are interested, the National Wildlife Health Center of the US Geological Survey produced an excellent video demonstrating the double method of euthanizing a bat, which can be used for training personnel (available online at https://www.usgs.gov/media/videos/approved-euthanasia-methods-bats-microchiroptera).

Disposal of bats and waste materials

After the bat is removed from the euthanasia chamber, it should remain closed until placed in a secure, well-ventilated, outside environment, to allow complete evaporation of the remaining isoflurane. Once the cotton ball is dry, dispose of it in regular garbage, and clean the chamber and tea strainer in hot water with an appropriate disinfectant, as determined through referencing scientific literature or consultation with experts. Dispose of other waste materials, such as syringes and disposable gloves, as biohazardous waste. Inhalant anesthetics can leave residues for days in euthanized animals (AVMA 2020). Therefore, carcasses resulting from euthanasia with isoflurane must be disposed of safely to prevent secondary toxicosis in other animals that may consume the dead bat. Safe disposal is best accomplished by collecting and submitting a euthanized bat to a wildlife pathologist (e.g., pathologists at the CWHC) for veterinary health surveillance purposes, such as documenting causes of health issues (see “Health Surveillance and Casualties”). Even if safe disposal is not required, such as when manual cervical dislocation is the only method used for euthanasia (i.e., isoflurane residue would not be present in the carcass), we also recommend submitting the euthanized bat for veterinary health surveillance purposes. If requested by personnel that have euthanized a bat, the health surveillance necropsy can include assessment of the head and neck to determine if an effective technique was utilized.

Unacceptable methods of euthanasia

Some approaches to euthanasia are no longer considered acceptable, because they ignore recent advances in technology and do not minimize risks to animal welfare, personnel safety, and the environment (AVMA 2020). In general, these unacceptable methods of euthanasia apply to most mammals, including bats, and involve the application of a range of inhumane physical killing techniques, such as air embolism, blow to the head, burning, decompression, drowning, exsanguination (unless blood is to be collected from the unconscious animal as part of the approved protocol), hypothermia, rapid freezing, slow chilling and freezing, and stunning (Sikes et al. 2011). Also unacceptable is the use of a range of chemical products, including cyanide, formalin, strychnine, chloral hydrate, various household products (e.g., bleach), and neuromuscular blocking agents (Sikes et al. 2011). Other inhalant and pharmaceutical euthanasia techniques specifically determined as unacceptable for bats include the use of carbon dioxide gas, T61, Ketamine, Telazol, Diazepam, Ketaset/PromAce, exhaust fumes, and inhalant compounds containing ether, nitrous oxide, or alkyl nitrites, such as lighter fluid, starter fluid, and air fresheners (Bat World Sanctuary 2010, Lollar 2018).

Health Surveillance and Casualties

Health of animals is highlighted as part of a guiding principle used by the CCAC (2021) to assess acceptable animal welfare. Examples of observable indicators of poor health include low body-condition score, traumatic injuries, visible lesions, evidence of inflammation, and morbidity and mortality rates (CCAC 2021). Records of these indicators can also provide insight on health issues and anthropogenic threats contributing to, or causing, population decline, and such evidence can be difficult to obtain, because the cryptic nature of bats makes it difficult to find sick or dead animals. Projects that involve capturing and handling bats, therefore, provide a unique opportunity to detect sick, injured, and dying bats, which can then be used as appropriate specimens for passive surveillance purposes to determine the cause of the identified health issue.

Occasionally, research protocols and techniques involving the capture and handling of wildlife can directly cause traumatic injuries and mortalities in the study subjects. For example, some species may be more susceptible than others to stress-related problems, such as capture myopathy in Northern Hoary Bats (Jung et al. 2002). To maintain high-quality welfare standards, the CCAC (2022) recommends establishing humane intervention endpoints, which are predetermined criteria that indicate when action must be taken to reduce suffering. These endpoints can be determined by informally consulting with scientific and veterinary professionals, referencing scientific literature, performing pilot studies or, if necessary, formally obtaining an expert opinion. Therefore, before beginning fieldwork, researchers should thoroughly investigate potential causes of injuries and mortality in their target species by reviewing the literature and talking to others who have previous experience capturing and handling the same species. The appropriate course of action and approved intervention endpoints should be clearly outlined in the project protocol, so that they are not open to interpretation if a circumstance arises for which humane intervention appears warranted. Approved humane intervention endpoints must be recognized and adapted as necessary in the event of expected and unexpected outcomes (CCAC 2022).

We recommend immediate suspension of a project if ≥2 casualties occur during fieldwork, until the investigators determine why these injuries or mortalities have occurred. It is, therefore, incumbent on those involved to ensure animals that are euthanized or subsequently die are necropsied (e.g., at a CWHC Regional Centre), to determine if anything related to the research procedure was the cause of death. This enables research protocols and techniques to be evaluated thoroughly to provide an evidence-based approach for changes and improvements that can prevent similar negative health outcomes (e.g., injury, illness, death) in future work. Thus, maintaining strong relationships with wildlife health specialists (e.g., CWHC), who can assist with the investigation of casualties and development of proposed mitigative measures, is essential. Lastly, post-mortem examination of dead bats enables new and emerging health issues to be identified, particularly infectious disease problems (e.g., WNS), and can lead to a better understanding of known diseases (e.g., rabies) for the protection of bat and human health.

Investigators should report unhealthy bats (see "Poor health") and those observed outside hibernacula in winter, including sick, injured, or dead bats, to local wildlife agencies to determine the best course of action. Often, agencies suggest submitting dead specimens to your local CWHC Regional Centre or other diagnostic laboratory for a complete post-mortem examination, usually at no cost. Ensure you follow instructions for submission, such as keeping specimens cool or frozen until shipped, but do not use formaldehyde as a preservative because it is a human carcinogen. A permit to ship specimens may be required because many provinces and territories have a "wildlife act" that regulates the import and export of animals, including bats. Please consult your federal, provincial, and territorial wildlife departments for policies and permits required for the legal importation and exportation of wildlife specimens and samples. If you have any questions about a sick or dead bat, you can call or email your local CWHC Regional Centre (https://www.cwhc-rcsf.ca/report_and_submit.php) to get the appropriate guidance or additional information.

Closing Remarks

These recommendations represent our collective experience and knowledge, as well as what can be learned from the literature. Our recommendations are not meant to be prescriptive; investigators and regulators must make decisions about best practices for their unique circumstances and assess specific research projects on a case-by-case basis. We acknowledge that some projects may require transporting bats, holding and caring for bats in captivity, and veterinary procedures that are more invasive than what we describe. Therefore, for guidance beyond the scope of this paper, we recommend investigators also consult CCAC (2003), Kunz and Parsons (2009), Lollar (2010, 2018), and Sikes et al. (2016).

We also recognize that decisions in the field often need to be made in response to unplanned events and can be stressful for people and bats alike. These guidelines are designed to help investigators develop preventative plans in anticipation of potential problems that may arise while in the field and to develop contingency plans to mitigate stress or injuries to bats. At the same time, we recognize that not all welfare issues can be anticipated; in such cases, practitioners should rely on their best judgement, drawing from information contained in this document, to mitigate or prevent negative outcomes. If an *a priori* plan is not in place for an incident that arises, immediately seek guidance from animal health specialists, bat experts, or management authorities.

Our recommendations are based on the best available information. We acknowledge that new information on best practices regarding capturing, handling, marking, and sampling bats will emerge. Many gaps in our knowledge exist, highlighting the need for empirical studies examining best practices. For example, better information on predation risk associated with the use of light tags, the applicability of the 5% rule across species of different body mass and morphology, and the impact and retention of bands and PIT tags will undoubtedly influence refinement or rejection of our recommendations. The use of antibiotic creams on wounds also warrants investigation. While these creams may appear beneficial, there may be unanticipated negative consequences, if the material is ingested or absorbed cutaneously, or antibiotic resistance develops. We, therefore, encourage research investigating bat welfare, to improve guidance on best practices and procedures.

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