Welfare and Handling Recommendations for Bat Censuses in Canada

Prepared for Parks Canada Agency

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Contributions

Krista J Patriquin: contracted by Mersey Tobeatic Research Institute (MTRI) to facilitate workshops and write the report.

Lori Phinney: contracted by Parks Canada Agency (PCA) and the Canadian Wildlife Health Cooperative (CWHC) to facilitate the workshops and provide in-depth input on the report, as well as to manage funds.

Scott McBurney: conceived of this project, secured funds, worked with other CWHC, Atlantic Region members to write the scientific literature review that informed this report, contributed to workshop discussions, provided in-depth input on all drafts, and wrote certain sections.

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CWHC, Atlantic Region: members authored the scientific literature review that informed this report and assisted with formatting and editing of document.

Canadian Bat Welfare Working Group: members were consulted during three workshops and individual discussions, when needed. They also provided feedback on earlier drafts of the report.

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Cover photo: *Myotis septentrionalis* in hand. Credit Jordi Segers, CWHC

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Executive Summary

Prior to initiating a project requiring direct handling of bats, it is essential that personnel receive first-hand training in bat capture and handling techniques from an experienced professional.

The goal of this document is to enhance bat welfare by providing a comprehensive set of guidelines for Canadian investigators and regulators with involvement in, and oversight of, those projects with a primary focus on censusing bats and monitoring bat health. We recognize that decisions in the field are often made "on the fly" and can therefore be stressful for those working in the field and bats alike. These guidelines should help investigators and regulators consider the welfare of bats during all stages of the fieldwork, develop contingency plans to mitigate stress, and avoid problems by preventative planning. At the same time, we recognize that not all incidents can be anticipated, in which case practitioners should rely on their best judgement drawing from information contained in this document.

This document is ***not*** intended to replace the 2003 Canada Council on Animal Care (CCAC) *Species-specific recommendations on: bats*. These current guidelines pertain specifically to "catch-mark-release" demographic censuses and surveys monitoring bat health. Investigators with more targeted research projects that extend beyond censusing bats (e.g., transporting, holding, and caring for bats in captivity; medical procedures) may also find this document helpful. However, it is important to recognize that some projects may require greater flexibility in applying research techniques that will allow the advancement of our understanding of bat biology and contribute in a meaningful way to conservation and management. As such, these recommendations are not meant to be prescriptive; investigators and regulators should use common sense when making decisions for their scientific objectives and unique conditions, and specific research projects should be assessed on a case-by-case basis in terms of best practices for bat handling and welfare. It is important to acknowledge that recommendations presented here will evolve, and therefore may be modified and superseded as new information becomes available. Therefore, practitioners are encouraged to consult this document for any new Federal/Provincial/Territorial regulatory information that pertains to field research on bats.

To ensure all relevant information can be easily accessed by all stakeholders (including those that may not have ready access to peer-reviewed literature), we provide a thorough synthesis of existing techniques and practices found in peer-reviewed and grey literature. To ensure knowledge not reported elsewhere is also included, we consulted practitioners with extensive experience working with bats across Canada, and from a variety of sectors (e.g., academia, Non-Governmental Organizations (NGOs), government, veterinarians, consultants, and animal welfare advocates). In addition, we have incorporated reviewers' comments on an earlier draft of this document provided by regulators from across Canada with jurisdictional responsibilities for bat conservation and management. Readers can expect to find updates to established techniques, where needed; introduction to new practices; and guidelines for the following: (1) Biosafety (vaccinations, personal protective equipment, decontamination), (2) Capture and removal from traps (mist-nets, harp traps, handheld traps, hand capture), (3) Restraint (bats in hand, holding bags and bins, number of bats per bag/bin, body mass, restraining devices), (4) handling and handling duration (time of night, local environmental conditions, season, species, morphometric and demographic data collection, torpor, and bats in poor health), (5) Provisioning bats, (6) Releasing bats, (7) Short- (water-soluble markers,

non-toxic hair dye, bee markers, hair removal, biopsy punches, light tags) and long-term marking techniques (bat bands, passive integrated transponders) and marking methods not recommended, (8) Biological samples (urine, milk, blood, biopsy punches, hair, fecal, ectoparasites), (9) Photography, (10) Euthanasia, (11) Health surveillance.

Because the number of anticipated captures must be specified on permit applications, we also provide an overview of the number of bats to anticipate with respect to: (1) Geographic region and climate, (2) Weather and season, and (3) Habitat. We also identify existing knowledge gaps.

To help investigators and regulators, we also provide a table outlining suggested decision thresholds for capture, handling, and holding bats. Additionally, we provide a series of images of the various practices outlined above. We also highlight existing knowledge gaps relating to potential impacts of current practices on bat welfare.

Investigators planning projects using techniques that extend beyond censusing bats and field monitoring of bat health, including transporting, holding, and caring for bats in captivity, medical procedures on bats, and methodologies beyond the scope of these guidelines, should consult one or more of the following: CCAC (2003), Kunz and Parsons (2009), Lollar (2010, 2018), and Sikes *et al.* (2016).

Message to Regulators

The purpose of this document is to provide:

- An overview of existing, updated, and new guidelines for the safe handling of bats.
- *Guidelines* specifically for catch-mark-release censusing and assessments of bat health.

This document is ***not***:

- A "how to" guide for conducting surveys (e.g., how and where to set up nets; how to measure forearms, tooth wear; how to sample for *Pseudogymnoascus destructans* etc), but rather a guide for how to mitigate any potential harm to bats (and practitioners) when using these methods. Readers looking for survey techniques can begin by consulting Inventory Methods for Bats: Standards for Components of British Columbia's Biodiversity (Resources Information Standards Committee (RISC), 2022) and sources cited throughout.
- Intended to replace the 2003 CCAC *Species-specific recommendations on: bats*.
- Intended to replace, but rather complement, guidelines already established in some regions (e.g., Handbook of Inventory Methods and Standard Protocols for Surveying Bats in Alberta (Vonhof, 2010); Inventory Methods for Bats: Standards for Components of British Columbia's Biodiversity (RISC, 2022)).
- *Prescriptive*
    - o we endeavoured to address all possible scenarios bat practitioners may face while conducting censuses and assessing bat health, but they may not apply to all applicants or projects.
    - o a case-by-case assessment will be needed for practitioners seeking permits for more targeted research questions that extend beyond simple censusing and bat health assessment. In this case, researchers should work closely with their institutional and/or organizational animal care committee.

Navigating the Document

- A table of contents is provided with links to help users quickly locate information most relevant to their needs.

- Also included is a table of decision thresholds for suitable trapping intensity, handling times, and holding times for different habitats, seasons, and bat demographics. This table contains a range of acceptable thresholds based on feedback from topic 8 experts across Canada.

    - More conservative thresholds represent the lowest risk to bat welfare.
    - Less conservative thresholds represent those that come with greater risk to bat welfare but may be warranted to successfully obtain the required data or results of the survey.

- In cases where existing practices are well documented, a brief overview may be provided, and readers are encouraged to consult the literature cited for additional details.

- In cases where the description for a technique or procedure is not yet published because new methods have been adopted or existing methods have been modified, additional details are provided.

1.0 Background

The current standard for safe care and handling of bats in Canada is the 2003 Canadian Council on Animal Care (CCAC) *Species-specific recommendations on: bats*. Since then, new survey tools for bats (e.g., PIT-tags) have become available and are widely used, while changes to improve existing tools and practices have also been made. As well, several Canadian bat species (*Myotis lucifugus*, *M. septentrionalis*, and *Perimyotis subflavus*) have since been listed as Endangered under the Species at Risk Act (SARA) due to dramatic population declines associated with widespread mortality from the fungal pathogen (*Pseudogymnoascus destructans*, *P.d.*) responsible for white-nose syndrome (WNS) (Environment and Climate Change Canada (ECCC), 2018). Additionally, the migratory species *Lasiurus cinereus, L. borealis* and *Lasionycteris noctivagans* have been assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as Endangered and recommended for listing under SARA as they have been negatively impacted through mortality associated with trauma from the expansion of wind energy developments across their range (Arnett and Baerwald, 2013; COSEWIC, 2022). As a result of these threats to Canadian bat populations, new types of surveys have been introduced, while the number of individuals and organizations seeking to better understand and protect bats have increased considerably. At the same time, recommendations for the safe handling of bats are 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). Meanwhile, organizations issuing permits for bat work are relying on the 2003 CCAC guidelines and there is no current timeline for revision to include new techniques (G. Griffin, personal communication). Also, those responsible for permitting may not have direct knowledge of bats and may rely on guidelines from other taxa to inform their management approach. As such, these wildlife professionals are less able to adequately critique applications and set appropriate guidelines to balance project goals with bat welfare. It is therefore our goal to provide an updated set of bat handling guidelines to help decision makers in those Federal/Provincial/Territorial departments with the jurisdictional responsibility for wildlife health as well as academics and other wildlife professionals working with bats adopt the best welfare practices. Additionally, it is our hope that these guidelines will help inform the CCAC when they revise their species-specific recommendations for bats.

To this end, the Canadian Wildlife Health Cooperative (CWHC) completed a literature review for Parks Canada (PCA) entitled *Literature review for the safe care and handling of bats in research and management within Parks Canada places and beyond* (Losada *et al.,* 2021). Subsequently, the Mersey Tobeatic Research Institute (MTRI) and Dr. Krista Patriquin consulted a committee of bat experts and practitioners across Canada (Canadian Bat Welfare Working Group, CBWWG) to gain valuable knowledge on new (and old) welfare and handling strategies that may not be reported in the peer-reviewed and grey literature. For a Canadian national perspective, practitioners with extensive experience in handling, welfare, and health of bats were selected to represent different regions across Canada (e.g., eastern, central, prairies, western, northern), as species and climate vary widely which could affect recommendations. Efforts were made to also represent different stakeholders (e.g., academics, NGOs, government, veterinarians, consultants, indigenous, animal welfare advocates), as their project objectives and methods can also vary. Together, the literature review and consultation process informed the creation of this document. Where possible, recommendations are supported with peer-reviewed literature. Otherwise, recommendations are based on the professional opinions of CBWWG members based on cumulative experience spanning decades and all regions of Canada.

2.0 Scope

This document is not intended to replace the 2003 CCAC *Species-specific recommendations on: bats*. Instead, we provide an update on established techniques, where needed, and introduce new best practices for investigators and regulators with the goal of enhancing bat welfare. Specifically, the recommendations outlined herein are intended primarily for "catch-mark-release" demographic censuses and surveys monitoring bat health as currently these are among the most common types of work requiring handling bats in Canada. According to Parks Canada Agency (PCA), 100% of applications (n = 22) submitted to their animal care committee from 2011–2021 for bat-related work in national parks and historic sites requested to conduct censuses involving capture and marking, as well as collection of non-invasive, typical biological samples (e.g., hair, tissue, fecal). Of these, only 14% also intended to answer specific research-driven questions requiring more extensive handling and invasive procedures. As such, we provide recommendations for a range of capture and marking methods. Detailed methods are provided for techniques that may influence bat welfare, while methods not relevant to bat welfare are not addressed. For example, methods for attaching a radio-tag are provided while methods for effectively tracking bats are not. As such, these guidelines do not address recommendations that may require long-term holding of bats, or projects involving captivity. Investigators planning projects that extend beyond censusing bats, including transporting, holding, and caring for bats in captivity, medical procedures on bats, and methodologies beyond the scope of these guidelines, should consult one or more of the following: CCAC (2003), Kunz and Parsons (2009), Lollar (2010, 2018), and Sikes *et al.* (2016). It is important to acknowledge that recommendations presented here will evolve, and therefore may be modified and superseded as new information becomes available. Readers are encouraged to visit the CWHC Bat Health Resources website to find the most up to date version of this living document.

3.0 General Considerations

Prior to initiating a project requiring direct handling of bats, it is essential that personnel receive first-hand training in bat capture and handling from an experienced professional that, in some cases, may include a veterinarian (e.g., euthanasia etc). Ideally a mentor will supervise personnel until proficiency in trapping and handling is demonstrated. If a knowledgeable mentor is not available within an investigator's organization, those requiring training could gain experience by volunteering with bat experts or participating in bat handling workshops.

For any project, the welfare of live animals must be carefully considered. The Animal Behavior Society's Guidelines for the Use of Animals (2022) recommends "for both scientific and ethical reasons, investigators studying free-living animals are expected to take precautions to minimize the imposition of fear, distress or lasting harm on individual animals, and to minimize the impacts of the study on the populations and ecosystems of which the individual animals are a part" (p.III).

The ethical framework of Russel and Burch's "Three Rs" can guide bat practitioners (Animal Behaviour Society, 2022; CCAC, n.d.). In the case of censuses, *reduction* and *refinement* should be prioritized as *replacing* live animals through other methods may not address the primary goals of a census. That said, advances in molecular analyses of guano may be of use (Guan *et al.,* 2020). The number of animals can be *reduced* by establishing a priori what data are needed to answer the project's objectives and/or hypotheses. Consequently, longer handling times for any given animal may be warranted when it reduces the total number of individuals impacted (Animal Behaviour Society, 2022). Investigators must therefore carefully balance the potential consequences of prolonging the acute stress of capture and handling on an individual in a single event to obtain the necessary data versus the cumulative stress that could occur if repeated captures are needed to acquire the same data. For example, projects involving capture of free-flying bats often require repeated sampling of sites because bat activity varies with weather conditions, prey availability, and reproductive season. *Refinement* refers not only to making further adjustments to minimize the project's activities on any individual's lifetime welfare, but also for a priori project endpoints (Animal Behaviour Society, 2022). In the case of bat censuses, investigators should monitor bat welfare and modify capture and handling procedures in real time as needed. They should establish, and specify in protocols, a priori procedural endpoints for: (a) time spent in traps, including the time it takes to remove bats from traps, (b) handling time during processing, (c) total holding time until release, which includes (a) and (b), and (d) euthanasia. When considering the Three Rs, investigators should also take into consideration species, sex, age, reproductive stage, and region (discussed later in this document).

In addition to obtaining approval from animal care committees, investigators must also obtain all appropriate permits and permissions from landowners and Federal/Provincial/Territorial departments with the jurisdictional responsibility for the management and conservation of bats. More than one permit may be needed and will depend on the species targeted (e.g., a Wildlife Collecting Permit and a Species At Risk Act permit may both be required for threatened or endangered species in some jurisdictions, while approval from a Animal Care Committee may also be needed), the proposed project (e.g., acoustic monitoring, capture of free-flying bats, roost counts, hibernacula surveys), and the land ownership status of the study area(s) (e.g., federal/provincial/territorial crown land, designated parks and protected areas, Parks Canada Heritage Areas, First Nations, private). For work on Parks Canada lands, investigators must apply for a Parks Canada Research and Collection permit (PCA, 2022). For work on other federal lands, investigators should consult the Species at Risk Act Permit System (Government of Canada, 2022). Provincial and territorial wildlife departments must be contacted to determine permitting requirements as they may each have their own Species at Risk legislation and Wildlife Acts. As a result, provincial and territorial regulators set their own guidelines and endpoints on permits according to the unique, situational, management and conservation circumstances for the species in their jurisdictions. In addition to obtaining the necessary permits, investigators planning to conduct work on private lands should contact landowners for their permission. Permits and permissions take time to acquire, so investigators should begin planning well in advance of their proposed start date. In some cases, permits may be required to purchase trapping (e.g., mist-nets) and marking materials (e.g., bands).

Investigators are encouraged to consult bat medical references (e.g., Lollar, 2010, 2018) and contact local wildlife health centres, licensed wildlife rehabilitators, or wildlife veterinarians familiar with bat health to determine available supports and recommended protocols in the event sick or injured bats are captured and/or unanticipated incidents harmful to bats are experienced. Contact information for these organizations should be provided to all personnel. If dead bats are encountered, or bats die/are euthanized during the project, they should be submitted to a CWHC regional centre or similar diagnostic lab for a post-mortem examination (free of charge at CWHC regional centres) to determine the cause of their sickness, injuries, or death (see 11.0 "Health Surveillance").

Personnel should be mindful of sampling procedures, species, and numbers of bats that have been approved in their permits. It can be tempting to collect data for colleagues or from non-target species, but this increases stress on bats and violates the conditions outlined in permits.

4.0 Biosafety

Bats are known reservoirs for pathogens infectious to other bats, as well as zoonoses and disease-causing agents for other animals and humans (Dutheil *et al.,* 2021; Joffrin *et al.,* 2018; Wibbelt *et al.,* 2009). For this reason, care should be taken when handling bats to prevent transmission of known infectious agents between bats and personnel working with them, as well as to mitigate the possibility for exposure to potential pathogens. Below we offer some guidelines, but personnel should follow appropriate biosafety procedures as directed by their permitting agency, which might include recommendations for specific vaccinations and personal protective equipment (PPE).

In Canada, the zoonotic pathogen of greatest concern to humans working with bats is the rabies virus (*Lyssavirus* genotype 1) because contracting the virus in unvaccinated individuals can be fatal (Government of Canada, 2018). Rabies virus is transmitted via bites and scratches from infected bats or introduction of infected bat saliva into open wounds or on to mucous membranes (Fenton *et al.,* 2020). Although the prevalence of rabies in wild bats varies by species and region across Canada, it is generally less than 1% in any given population (Segers *et al.,* 2021). Moreover, hundreds of thousands of bats live in thousands of human occupied buildings across Canada but the incidence of rabies spillover to people is low (Government of Canada, 2015). Since 1970, there have been nine human rabies deaths in Canada, hence rabies fatalities are rare (Fenton *et al.,* 2020). Interestingly, seven of these deaths were caused by bat rabies virus variants. If people come into contact with a suspected rabid animal, they should immediately call their local medical officer of public health or physician, report their interaction and determine if further medical attention is required (British Columbia Center for Disease Control (BCCDC), 2022).

Bats with rabies may appear asymptomatic, or they may display either the 'furious' form where they are exceptionally aggressive, or the 'dumb' form where they are lethargic and uncoordinated (BCCDC, 2022; Fenton *et al.,* 2020). However, when considering clinical signs of clumsiness or lethargy, care should be taken not to mistake this for a torpid or listless bat (see 6.5.7 "Torpor" and 6.5.8 "Poor health"). Rabid bats can have difficulty swallowing, display rigid, outstretched wings when held, have difficulty flying, and might 'flutter' on the ground when released (Center for Disease Control (CDC), 2022; Global Alliance for Rabies Control (GARC), 2014).

Histoplasmosis is another zoonotic disease associated with bats in Canada (Figure 1, Canadian Center for Occupational Health and Safety (CCOHS), 2022). This fungal disease is caused by *Histoplasma capsulatum*, and infection can occur when humans are exposed to aerosolized fungal spores present in bat guano (and bird droppings), or soil contaminated by their excreta. Although infections are rare, the risk is typically limited to enclosed spaces where large accumulations of feces or contaminated soils occur, particularly in Quebec, Ontario, and Alberta (though records also exist in Saskatchewan and New Brunswick; Allard *et al.,* 2014; Anderson *et al.,* 2006; CWHC, n.d.; Dingle *et al.,* 2021; Nicolle *et al.,* 1998; Tyre *et al.,* 2007). The disease primarily affects the lungs causing pneumonia, but more serious generalized life-threatening infections can also occur.

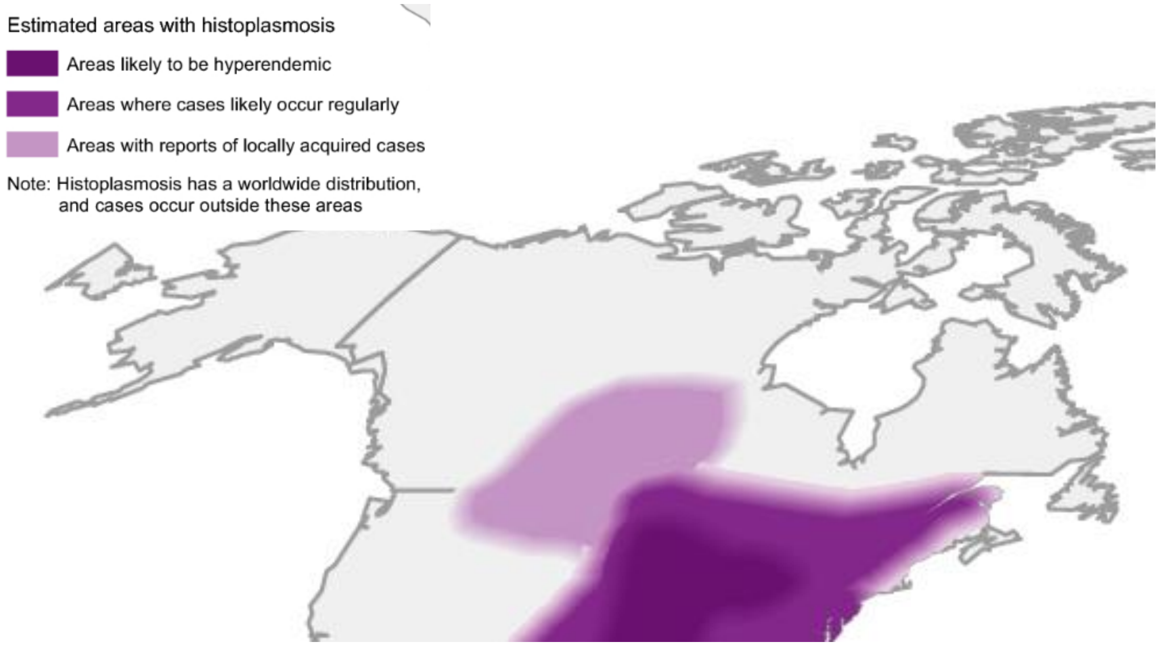


Figure 1. Map of northern North America estimating regions most likely to have histoplasmosis based on literature review (adapted from Ashraf *et al*., 2020.)

Transmission of fungal pathogens among bats is also of concern. Of great significance in North America, including Canada, is the spread of *P.d.* that causes WNS in bats. The introduction of the fungus and its spread in North America may be related to anthropogenic activities (Blehert, 2012; Shelley *et al.,* 2013), so care must be taken to prevent human movement of *P.d.* while capturing and handling bats. For detailed information regarding WNS decontamination protocols, visit:

http://www.cwhc-rcsf.ca/bat_health_resources.php#white-nose-syndrome

https://www.whitenosesyndrome.org/

Like all animals, bats are also infected with their own viral, bacterial, and fungal pathogens, as well as ectoparasites (Avena *et al.,* 2016; Czenze and Broders, 2011; Irving *et al.,* 2021). While most of these are host-specific and therefore pose no risk to humans, they could all potentially impact bat health. Care must therefore be taken to prevent transmission of pathogens and parasites between bats while they are being captured, held, and handled.

While the diseases noted above can be significant, prevention of human infections and spread of infections during capture and handling procedures is relatively straightforward when basic biosafety protocols are followed. Therefore, the recommendations below highlight the techniques to limit the movement of infectious organisms and parasites from bats to humans (i.e., zoonotic transmission), between bats, and from humans to bats (i.e., zooanthroponotic or reverse zoonotic transmission).

Although bats in Canada are not known to be reservoirs for SARS-COV-2, or to be susceptible to the virus, an abundance of caution (i.e., appropriate PPE) is recommended to limit potential reverse zoonotic transmission of this viral pathogen from infected humans to bats (Cook *et al.,* 2022; CWHC (CWHC, 2021). The CWHC has recently published SARS-CoV-2 protocols for handling bats. At the time of writing this document, biosafety and biosecurity recommendations are frequently evolving in consideration of this emerging disease that is becoming endemic. Readers should therefore consult the CWHC for the most up-to-date guidelines related to bats and SARS-COV-2. Feedback is also most welcomed (info@cwhc-rcsf.ca).

4.1 Vaccinations

While vaccination guidelines may vary across provinces and territories, all personnel working with bats must have pre-exposure rabies prophylaxis vaccinations and have their serum tested to ensure they have an adequate rabies antibody titre following Health Canada's rabies immunization guidelines (CCAC, 2003; Government of Canada, 2015; Vonhof, 2006). It is worth noting that careful planning is needed for pre-exposure rabies immunization as this may involve a series of doses over a span of a month (Government of Canada, 2015). Personnel who handle bats should have their rabies antibody titre checked at least once every two years (Rao *et al.,* 2022; though annually is preferred if possible) and receive a booster shot if their titer falls below levels recommended by the World Health Organization (Government of Canada, 2015, CDC 2022). In the event a vaccinated person is bitten or scratched, they should thoroughly wash the area with soap and water, after which they should apply an antiseptic (e.g., Betadine or ethanol). They should also report this direct contact with a bat to their physician, local medical officer, and/or Public Health Agency of Canada. The Provincial Departments of Health will give the appropriate medical guidance, including further post-exposure immunization prophylaxis, as well as use of rabies immunoglobulin (RabIg) in those individuals without appropriate previous rabies immunization (Government of Canada, 2015; Public Health Ontario, 2017). In these cases, the bat should be kept for further examination and is typically euthanized (see 10.0 "Euthanasia") and submitted for rabies testing. It is therefore extremely important that personnel are aware of this eventuality and take appropriate precautions to avoid being bitten or scratched so that normal, healthy bats are not killed unnecessarily to protect human health.

Note: When considering post-exposure prophylaxis, treatment with RabIg is **not normally** required for someone who has been previously appropriately immunized and has a protective antibody titre (Government of Canada, 2015; Public Health Ontario, 2017). Additionally, in previously appropriately immunized individuals with a protective antibody titre who require post-exposure prophylaxis, **only two** vaccine doses are recommended on day 0 and 3 days later. That said, please consult your physician and/or provincial or territorial Public Health for guidance (e.g., Government of Canada, 2015; Public Health Ontario, 2017).

4.2 Personal protective equipment

Historically, there has been little mention of the need for PPE when handling bats. However, PPE is now strongly recommended due to the increased awareness of the potential for zoonotic and zooanthroponotic transmission of known and unknown pathogens, as well as transmission of diseases between bats during capture, holding and handling. Detailed PPE guidance can be found on the CWHC website under Resources (CWHC, 2022).

4.2.1 Gloves

Gloves should always be worn when handling bats to prevent bites, scratches, and pathogen transmission (Couper, 2016). The appropriate gloves for handling bats depend on both the species being handled and personal preference. In areas where a variety of species with different biting propensity and strength may be encountered, personnel should keep on hand several glove types with spares so they can be changed as needed. Well-fitted leather gloves are recommended to prevent bites and scratches (Figure 2). Other reusable glove types that offer adequate protection include golf, riding, and gardening gloves (Couper, 2016; Hooper and Amelon, 2014; Vonhof, 2006). Disposable surgical-type gloves (latex or nitrile) worn over reusable gloves is also recommended to limit pathogen transmission to personnel and between bats (Figure 2; Couper, 2016; CWHC, 2021, 2022). Note, bats may be allergic to disposable latex gloves (CWHC, n.d.). Bats can have difficulty freeing their teeth if they have bitten into nitrile gloves and may lose teeth as a result (C. Lausen, pers. comm. 2022). At the same time, reusable leather gloves may inhibit dexterity, especially when working with smaller bats. Some handlers therefore prefer to wear a disposable glove on the dominant hand to improve dexterity while holding the bat in the non-dominant hand with a reusable glove covered by a disposable glove. For smaller species (<10g) with lower bite strength, wearing two disposable gloves on each hand (i.e., 'double gloving') improves dexterity and prevents bites and scratches.

Figure 2. Bat held in leather gloved hand (left - Krista Patriquin) and nitrile gloved hand (right - Lori Phinney).

4.2.2 Masks

We suggest personnel follow the latest CWHC recommendations related to SARS-CoV-2 and masking guidelines (e.g., N95 mask). Respirators with a high efficiency particulate air (HEPA) filter capable of filtering 2-micron particles are recommended when there is risk of exposure to *H. capsulatum* spores while working in building-roosts and caves (CCOHS, 2022). It is unknown if respirators without exhalation valves help to protect bats from potential exhaled human pathogens.

4.2.3 Clothing

Outer clothing, such as coveralls, is recommended primarily to reduce the risk of transfer of pathogens between sites, but also to protect personnel and bats (CCOHS, 2022; CWHC, 2021, 2022). Suitable clothing should have long sleeves to cover the forearms to the level of gloved hands to prevent bites or scratches on exposed skin. Disposable suits should be properly disposed of, but coveralls can be washed (see 4.3 "Decontamination").

4.2.4 Footwear

As with outer clothing, footwear should be decontaminated according to guidelines below. Alternatively, outer coverings for footwear can also be used.

4.2.5 Topical lotions and sprays

Personnel may need to apply sunscreen and insect repellants when working in the field. In these instances, hands should be thoroughly washed with soap and water after application to ensure bats do not ingest these chemicals. Similarly, personnel should wash hands after touching any area that has been treated with chemicals. Alternatively, lotions and repellants may be applied with bare hands prior to putting on gloves. Prior to reapplying sprays, be sure to remove gloves and move a safe distance away from traps, processing stations, and areas where bats are held.

4.3 Decontamination

Before moving between different sites, any surface or equipment that may have come into contact with bats, roost surfaces, or hibernacula substrate should be disinfected, and in some cases sterilized, including headlamps, PPE, nets, traps, holding bags, restraining devices, processing materials, and cameras. Previously used equipment and materials should not be used across provincial, territorial, or international borders (RISC, 2021). To further minimize the risk of pathogen transmission, it may be advisable to have separate equipment and materials dedicated to specific regions or sites.

Detailed guidelines are available for decontaminating trapping and netting equipment, clothing, and footwear (CWHC, 2022), and processing materials (Iowa Department of Natural Resources (IDNR), 2016). These sources may also be consulted for suitable decontamination products available in Canada. Below is an overview of general recommendations for decontamination in different contexts.

Bats captured from the same summer colony:

1. Keep used and unused materials and equipment separated at all times.
2. Maintain several pairs of leather handling gloves to use between nights.
3. Before leaving a site, clean and disinfect all non-submersible survey equipment (e.g., cell phones, processing table, mist-net poles, harp traps, clip boards, calipers, rulers, etc.) and head lamps. with disinfectant wipes or sprays. If personnel enter roosts or hibernacula to capture bats, boots should also be disinfected with wipes or sprays. Be sure to remove all dirt from boots prior to disinfection. Where possible, rinse gear with clean water after disinfection and allow to dry.
4. Before leaving a site, place all used submersible equipment and field gear (nets cloth bags, and outer clothing) in plastic bins or bags. Once offsite, place contents in a washing machine (if available) with scent free soap in hot water and place in dryer (if available). If a washing machine and dryer are not available, fill the bins with hot water ($\geq 55^{o}$C), soak the contents for 20 minutes, and allow to air dry. Alternatively, contents can be placed in a large pot (dedicated solely to decontamination) with water and heated over a stove or other appropriate heat source until appropriate water temperature is attained.

Free-flying bats in summer, <u>follow all above guidelines</u> as well as:

5. Change disposable gloves between bats, if possible. Alternatively, wipe down gloves 70% isopropyl alcohol wipes between bats and allow to dry.
6. Clean and disinfect bat processing equipment (e.g., cell phones, calipers, rulers, etc) between bat captures, if possible.

Free-flying bats in winter and bats in hibernacula, follow all above guidelines as well as:

7. Disposable coveralls (Tyvek suits) are recommended. Before leaving a site, used suits should be sprayed with disinfectant, sealed in a plastic bag, and returned for incineration or disposal.
8. Washable coveralls are also acceptable and should be disinfected following Step 4 above.

5.0 Capture and Removal from Traps

5.1 General guidelines

Capture of free-flying bats normally takes place on calm nights with no precipitation, especially when using mist-nets. Bats can become severely entangled in nets when strong winds cause the tier panels to billow. Additionally, when nets are moved by the wind, they are more easily detected and avoided by bats, reducing the opportunity for their capture. When trapping in the rain, bats may use additional energy reserves to keep warm if they get wet and/or cold (but see 6.5.7 "Torpor"), which should be avoided if possible.

To improve capture success, it is recommended that personnel keep noise levels (including voices) to a minimum when checking traps, removing bats from traps, and handling bats. It is also advised to keep light levels to a minimum when checking traps. Some investigators prefer to use headlamps with a red-light filter or plastic cover instead of white light.

Personnel may consider carrying a means to monitor time between trap checks, captures, and removal of bats. Time of capture for each bat, or group of bats, should be noted (e.g., on the holding bag, in a field book, or on a datasheet). Reminders (preferably vibrating) of approaching release times should be set in the event personnel are overwhelmed and preoccupied by the number of captures and/or processing requirements.

Depending on the specific project, investigators may wish to limit repeated visits to the same site as bats may begin to avoid an area (Marques *et al.* 2013) or switch roosts when repetitively captured (Lewis 1995, Luo *et al.* 2012). When repeated visits are necessary to catch free-flying bats, consider separating visits by 1–7 nights, unless nets are placed in new locations (e.g., different flyways, bodies of water; Table 1; RISC, 1998, 2021). The costs of disturbing bats in roosts are not well known; in some instances, bats may continue to use the same roost after disturbance (Ferrara and Leberg, 2005), others may switch roosts (Lewis 1995), and still others may delay emergence times (Ancillotto *et al.,* 2019). However, the real costs to survival and reproductive success of remaining at a roost after a disturbance or roost switching are not known. Therefore, as a precautionary measure, investigators may choose to limit repeated visits to roosts, particularly known maternity roosts during parturition and nursing, and to separate roost visits by 3–30 nights (Table 1). Acoustic monitoring of known roosts may provide a reliable alternative to assess roost occupancy (Froidevaux *et al.* 2020).

Adequate personnel should be available to remove bats quickly and safely from nets and traps (hereafter referred to collectively as 'traps') and to subsequently (or simultaneously) process bats.

The minimum number of personnel will depend on several factors, including experience, capture method, number of traps, and number of anticipated captures (see 5.4 "Number of anticipated bats" for a discussion on how this varies with geographic region, habitat, and ambient conditions), as well as the data and samples to be collected from the target species according to the approved research protocol. Generally, it is good practice and safest to work with a partner in the field. We recommend a minimum of two people for conducting censuses to ensure sufficient personnel are available to check traps, as well as process and release bats. Having three or more personnel may help facilitate the required decontamination procedures during bat handling.

Bat detectors can help monitor general bat activity of an area and, to some extent, identify species presence. Active detectors can provide immediate feedback on bat activity in an area which may help inform frequency of net checks. Investigators can choose from models that vary in features such as sound output, a screen to visualize calls, headphone connectivity, or the ability to record calls (see Appendix I. Bat Capture Survey Equipment Suppliers). It is important to note that acoustic activity does not predict captures as detections could be from bats in nearby roosts or flying close by.

5.2 Experience

The time required to remove bats from traps will depend on trap type, individual bat behaviour, and degree of entanglement. Removing bats from mist nets can be challenging compared to other capture methods, such as harp traps and hand nets (but see 6.0 "Restraint, handling, and release"). Experienced personnel can normally remove a bat from a mist-net within 5–10 minutes (usually much less), but when a bat is more entangled, it can take longer. Once the bat is in captivity, experienced personnel can normally collect the basic morphometric and demographic data within 10–20 minutes (also usually much less). Longer handling times may be required for marking bats (see 7.0 "Marking") and when collecting biological samples (see 8.0 "Biological Samples"). It is common for inexperienced personnel to be slower and more cautious when removing a bat from a net and processing it (~double the times noted above), as such they may need oversight and assistance from experienced investigators if their handling time exceeds the maximum recommended time (Table 1). Experience should therefore be considered when estimating the number of required personnel, as well as endpoints for closing traps and releasing bats (see 6.5 "Handling duration")

5.3 Capture methods

5.3.1 Mist-nets

Deploying mist-nets (Figure 3) can take time, especially for inexperienced personnel, which should be considered when selecting the number of nets to deploy in an area. Normally, mist-nets are opened shortly after dusk to minimize captures of crepuscular birds. Nets should be clearly marked with signage and reflective tape placed on the poles when placed in areas with high pedestrian traffic, and potential bike, ATV, and other vehicular traffic.

It is recommended to check open mist-nets every 5–30 minutes to minimize capture stress (Table 1), risk of exposure to predators, and/or injury (RISC, 1998, 2021; Edwards *et al.,* 2022; Sikes *et al.,* 2016). A variety of species, including deer, frogs, owls, and fish, have attempted to prey on bats in mist-nets (CBWWG, pers. obs.). Domestic cats may also pose a risk to captured bats. Where possible, it may be helpful to request homeowners keep cats indoors for the night when trapping on their properties. Checking too frequently (e.g., every few minutes) may deter bats from approaching the net, while infrequent checks can lead to an unmanageable number of captured bats, resulting in

Table 1. Suggested range of 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 suggestions. Less conservative thresholds represent those that come with greater risk to bat welfare but may be warranted to successfully conduct a survey, based on expert suggestions. For more details, please consult relevant sections throughout.

Category	More conservative	Intermediate	Less conservative
Time Between Trapping			
Free-flying bats in the same location*	7 nights	4–5 nights	1–2 nights
Free-flying bats in same area with nets in different place*	5 nights	1 nights	0 nights
Maternity colony	≤30 nights	5–10 nights	3 nights
Night roost	≤30 nights	5–10 nights	3 nights
In hibernacula	1–2 times/season†	30 nights	0–1 nights
Free-flying bats outside hibernacula during swarming**	1–2 times/season†	14 nights	7 nights
Frequency of trap checks			
Mist-net checks	5 min	10–15 min	20–30 min
Harp traps	30 min	1–3hrs	1–2 times/night
Harp trap at maternity roosts	Continuous to 15 min at emergence, then 60–90 min thereafter		
Handling times			
Max 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
Holding times (summer)			
Non-target species	Basic data and release immediately		30 min
Any bat in poor condition	Basic data and release immediately		30 min
Bats in distress or at risk of morbidity or mortality doe to capture	Release immediately		

*i.e., trail, forest edge, etc.
**Same individuals rarely captured within 1–2 weeks (C. Lausen, pers. comm.)
† e.g., at beginning and end of season

Table 1. (continued)

Category	More conservative	Intermediate	Less conservative
Holding times (summer)			
Target species in good condition - within first hour of sunset			
Females with attached pup(s)	Basic data and release immediately		30 min
Late pregnancy females	Basic data and release immediately	30 min	1 hr
Nursing females (pups not attached)	1 hr		2 hrs
Early pregnancy females	1 hr	2–3hrs	4 hrs
Adult males, non-parous females, volant juveniles, post-lactating females	1 hr	2 hrs	4 hrs
Target species in good condition - between sunset and sunrise			
Females with attached pup(s)	Basic data and release immediately	30 min	1 hr
Late pregnancy females	Basic data and release immediately	30 min	1 hr
Nursing females (pups not attached)	1 hr		2 hrs
Early pregnancy females	1 hr	2–3 hrs	4 hrs
Adult males, nonporous or post-lactating females, volant juveniles	1 hr	3 hrs	4 hrs
Target species in good condition - within one hour of sunrise			
Any bats still being held (nets should be closed)	Basic info and release immediately		30 min
Holding times (not summer)			
Spring[1]	≤ 1hr BUT see [1]		
Fall[1]	≤ 1hr BUT see [1]		
Winter[2]	1 hour BUT see [2]		

[1]Depends on factors such as nightly temperature (decrease time if below optimal foraging temperatures reported in a region, as bats will have a smaller window to forage), use of torpor in captured individuals (if not going torpid will burn through fat while being held, so need to provide warmth if going to keep in captivity; if torpid, then keep up to 4hrs), and body size (smaller bats = higher surface area to volume ratio so lose heat faster/use more energy, even when torpid, so shorter holding time and/or heat source needed if keeping in captivity)

[2]1 hour, and bat must be provided exogenous heat during the entire time in captivity so that it expends minimal fat reserves on maintaining a normothermic body temperature while aroused from torpor during handling.

increased stress in the bats and personnel removing them as well as the possibility of bats becoming more entangled due to prolonged periods in the net. Bat detectors deployed near nets can provide an indication of activity, which can be used as a guide for when to check nets (i.e., high activity = more frequent checks). Baby monitors can also be used to detect bat captures, as bats often produce audible vocalizations when trapped. If left unattended too long, bats may also chew themselves free from nets, resulting in lost data and damaged nets. It is advisable to frequently check nets placed just above water to prevent risk of bats drowning (RISC, 1998).

Figure 3. Triple-high mist-net (Jordi Segers).

The number of mist-nets that can be safely deployed depends on number of personnel, anticipated time to remove bats (see 5.2 "Experience"), and anticipated capture rate (see 5.4 "Number of anticipated bats"). If most personnel have little or no experience removing bats from mist-nets, it is highly recommended that the number of mist-nets be limited until personnel become more technically proficient. Otherwise, it is common practice to deploy as many mist-nets as can be checked within 5–30 minute intervals while considering travel time between nets and time to remove bats from nets, as well as anticipated capture rate (see 5.4 "Number of anticipated bats"; RISC, 1998, 2021).

When checking nets, personnel should avoid crawling under nets to reach the other side as they can become entangled and therefore risk accidental tension on the nets, which could injure bats. For each tier, it is often best to begin with the least tangled bats, especially for less experienced personnel. This reduces the risk of further entanglement and decreases handling times. However, bats at risk of injury or that may be especially difficult to remove should be given the highest priority for immediate removal. If many bats have been captured in one net, it is recommended to begin removing bats from the lowest tier, which should then be closed as soon as it is free of bats to prevent additional captures until the entire net is free of bats. With enough personnel, it may be possible to carefully tilt a net closer to the ground by removing one or both pole(s) so that another person can reach bats caught in higher tiers. However, this should be a last resort and used with extreme caution to ensure further tension is not placed on the net which could potentially injure bats. Step ladders (or makeshift stools from field gear or objects in the environment) may also be helpful where logistically feasible and safe for personnel to use. Extreme caution should always be used to ensure additional net tension is not placed on captured bats and/or that they do not become increasingly entangled. All else being equal, it is advisable to remove target species first to reduce risk of escape when multiple species are captured at one time.

Bats should be removed from a net in the opposite direction from which they entered, so the last parts of the bat that entered the net are removed first (Battersby, 2010; Hoffmann *et al.,* 2010). Normally this can be done by locating the exposed tail membrane and removing the net by working from back to the front. While holding the tail membrane in the non-dominant hand, the net can be carefully removed with the dominant hand. When disentangling a bat, the freed parts of the body should be carefully grasped in the non-dominant hand to prevent their re-entanglement or entanglement. This is especially important for the feet as bats constantly try to grasp surfaces and their feet tangle and re-tangle easily. Some mist-netters prefer to use a bat bag to grasp the entangled bat, which may reduce stress, while exposing areas that are currently being freed. Care should be taken when removing strands of net from the wings to avoid breaking the delicate finger and arm bones (phalanges, metacarpals, humerus, radius, and ulna). A crochet hook may be helpful for removing any pieces of net caught in a bat's mouth (Figure 4; RISC, 2021). A plastic toothpick found in many utility knives may also be used and has the added benefit that it can often fit under a tight net strand to then slip the strand carefully over the tangled portion of the body. Small scissors or a dulled seam ripper are essential as they can be used to cut net strands to quickly remove bats that have been markedly entangled, especially if more than 20 minutes have passed since attempting their removal. After the bat is out of the net, ensure none of the netting is wrapped around a leg or wing, or strands remain embedded in the fur or caught in the mouth of the bat (Finnemore and Richardson, 2004). Personnel should also check under nets for bats that may have fallen to the ground.

Bats often bite and hold on to nets and gloves. When this happens, take care not to react by pulling back or making sudden moves as this may injure the bat or break its canine teeth. In addition, it is not recommended to forcefully remove the bat by pulling on the scruff of its neck or muzzle. A common solution that worked well in the past was to blow on a bat's face, which caused it to open its mouth and let go of the net. However, recently this practice generated some concern due to the possibility of SARS CoV-2 zooanthroponosis. Some investigators have therefore used the following alternatives:

1. Removal of tension from the net causing a bat to release its grip.
2. Compressed air cannisters to blow on a bats face, but a test spray should be performed to gauge distance, force, and direction of spray.
3. Large bulb air brushes used for cleaning camera equipment to blow on a bats face.
4. Gently tapping a bat on the head.
5. Using a thumb or finger, apply gentle pressure on top of the bat's head and slide to the base of the neck, causing the head to tilt back and the bat to release its grip (Figure 5).
6. As a last resort, gently opening the bat's mouth with plastic forceps, a cotton swab, or similar object (Hoffmann *et al.* 2010).

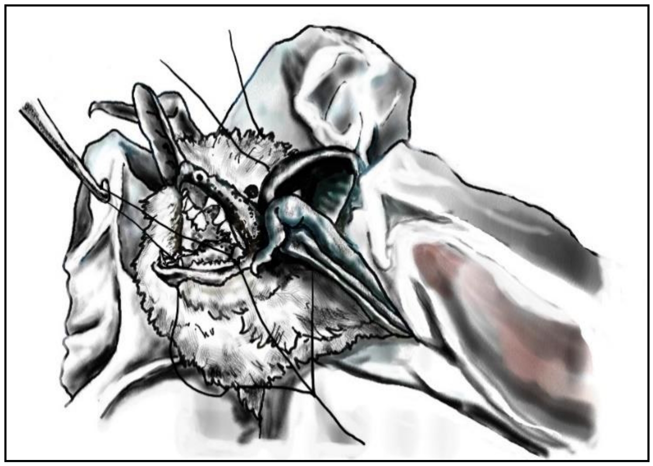


Figure 4. Crochet hook to remove mist net from bat's mouth (from RISC, 2022; L. Andrusiak).

Figure 5. Carefully sliding thumb from top of the bat's head to the base of the neck, causing the head to tilt back. Used to encourage bat to release its grip and to examine tooth wear (Krista Patriquin).

When personnel become overwhelmed by the number of captured bats, any open nets without any bats should be immediately closed by collapsing all tiers. Tiers should never be closed if they contain bats as they will become further entangled (see above). To prevent accidental captures in closed tiers, use a piece of string or flagging tape to tie tiers together at several points along the length of the net. When animal handling and processing times are expected to exceed normal maximum holding times (Table 1; see 6.0 "Restraint, handling, and release"), it is recommended that nets remain closed until personnel can remove and process the most recent captures. It is advisable to close all nets 30–60 minutes before dawn to allow time to remove and process bats, and to prevent capture of crepuscular birds.

Note: mist-nets may be made of either polyester or a much thinner monofilament nylon. Monofilament nets may provide higher capture success because they are not as easily detected by bats. However, bats may also become more entangled in monofilament nets making it more difficult to remove bats compared to those caught in polyester nets. As such, investigators using monofilament nets should take this into consideration when considering the number of nets to deploy and endpoints for net-checks and removal from nets. That said, some investigators have noted that at least some species (e.g., *Myotis lucifugus*) become less tangled and easier to extract in monofilament nets (J. Wilson, pers. comm., 2022).

5.3.2 Harp traps

Harp traps are recommended when it is anticipated that many bats could be captured in a short period of time, such as near a roost or hibernaculum (Figure 6). In these instances, it is not recommended to leave traps unattended as bats may get overcrowded and aggressive, with subsequent fighting and increased potential for injuries. Harp traps are also useful because they can be placed in narrow flyways, may require less frequent monitoring, and may be left unattended for extended periods. However, it is advisable to check traps using shorter intervals to prevent overcrowding and the potential for predation as well as so captured bats in poor health, pregnant or nursing females, females with pups, and non-target species can be quickly processed and released (see 6.5.8 "Poor health" and 6.5.6.5 "Reproductive status"). If harp traps are being placed at or near roost exits, there is a significant possibility that many bats will be caught soon after emergence. The overcrowded bats can fight and/or become injured, and they can also attract predators or inquisitive humans. In such circumstances, traps should not be left unattended for extended periods and instead should be regularly checked. Also, weather can become inclement throughout the night. However, the interior of most harp trap bags is lined with a plastic shield to protect bats from inclement weather. Opportunistic predators (e.g., snakes, rodents) may be able to access captured bats in harp trap bags. As such, it is advisable to check traps at least once an hour (United States Fish and Wildlife Service (USFWS), 2016). This interval can likely be extended by several hours (1–3 hours, up to twice a night) in areas known to have low activity, unless trapping when females are likely in late pregnancy or nursing. The number of harp traps that can be safely deployed depends on how frequently they will be checked while taking into consideration travel time between traps as well as handling and processing times. If the total anticipated processing time will exceed the recommended holding time, harp traps should either be moved from the flight path or tipped over to lay flat on the ground until most bats are processed and released. Removing the harp trap holding bag may also preclude captures, but bats may hit the trap and fall to the ground where they may have difficulty gaining lift to fly and may get trampled or preyed upon.

Another advantage of harp traps is bats can be removed relatively quickly and easily. That said, care should be taken when grasping a bat to ensure the wings are folded and nails are not hooked into holding bag's fabric before removing it. Bats should never be pulled out by their forearms or toes. As with mist-nets, personnel should consider total holding time in traps, and handling and processing time for release endpoints (see 6.5 "Handling duration").

In areas with a high capture rate, it may be advisable to replace or disinfect harp trap bags periodically throughout the night to minimize transmission of pathogens and parasites between captured individuals.

Figure 6. Harp trap (left - Tessa McBurney; right – Brock Fenton).

5.3.3 Handheld traps

Handheld traps (hand nets), such as insect nets with very fine mesh or cloth may be used to capture bats from walls or ceilings in roosts and hibernacula (Figure 7). To prevent bats from escaping, line the inner perimeter of the net with plastic, similar to a harp trap bag. Home-made traps (Kunz and Kurta, 1988; Figure 8) may be useful at roost exits. It is not recommended to use hand nets to catch free-flying bats due to the potential for injuries if bats collide with the hand net's edges (CCAC, 2003; Jackson, 2003). If such a strategy is employed, it is best to approach the flying bat with the net from behind rather than head on (CCAC, 2003). When catching a single or group of bats as they roost, approach the bat(s) from below and quickly cover them with the hand net, ensuring not to pin bats between the outer rim of the trap and roosting surface; bats will then fall or fly into the net. The non-dominant hand can then be used to pinch the top of the net closed to prevent bats from escaping, and the dominant hand can be used to carefully reach through the closed opening to remove the bat(s). Care should be taken to ensure wings are folded, and thumbs and toes are carefully unhooked rather than pulled from the net (Finnemore and Richardson, 2004).

Figure 7. Hand net (Avinet)

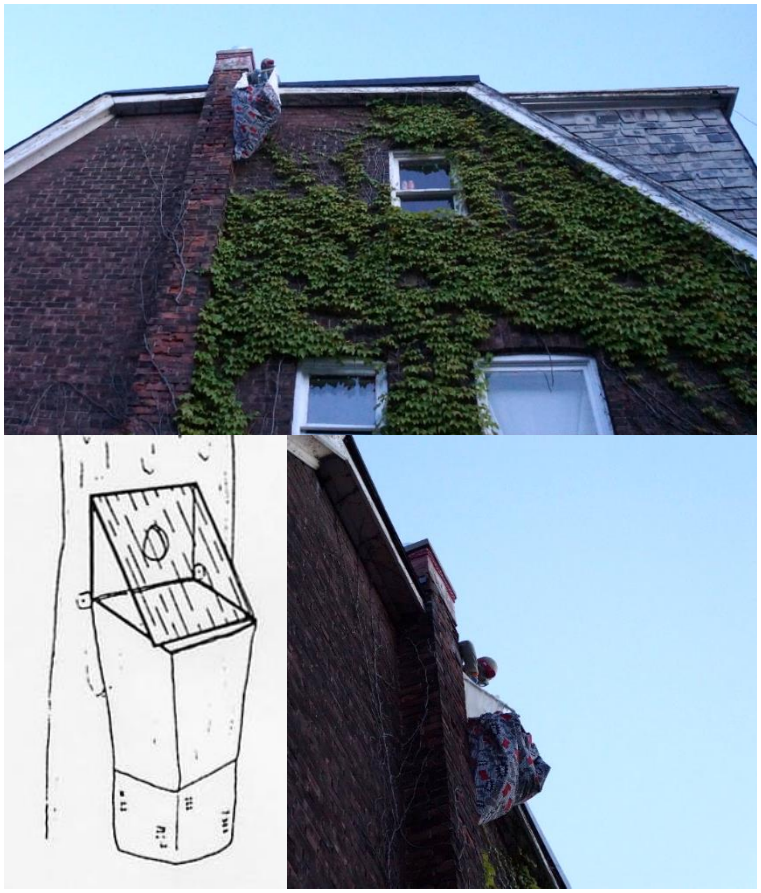


Figure 8. Home-made roost trap (bottom left from Kunz and Kurta 1998; top and bottom right - Krista Patriquin).

If capturing bats in roosts (e.g., in buildings), it may be helpful to do so during the day. This way bats can be processed during the day and released in time to forage in the night. In this case, bats should be returned directly inside the roost after processing or released at night at the roost site. If capturing bats exiting roosts, ensure the net or trap makes a tight seal around the exit (if possible). It is best to set up the trap at least 30 minutes prior to dusk and personnel should be extremely quiet as they wait for bats to emerge. When a potential threat is detected at a roost, bats will delay emergence until the perceived threat is gone (Ancillotto *et al.,* 2019). If bats do not emerge within 1 hour after dusk, the trap may be removed so bats can emerge to feed. Alternatively, traps can be moved away to a location near the roost exit and then placed over the exit again once bats begin to emerge, taking care to ensure that bats do not get crushed in the process.

5.3.4 Hand capture

When accessible, bats in roosts may be captured by hand (CCAC, 2003). Bats may also be extracted from tight crevices using long, blunt/padded tissue forceps (Sikes *et al.,* 2016) while taking care to avoid bruising, abrasions, and broken bones. This can be achieved by gently pulling the forearm until the bat starts to move in the desired direction and gradually pulling the bat closer by repeating this action until the bat has been removed from the crevice. Extreme care must be exercised with this technique to avoid potential injury; and if a bat shows resistance, the attempt to extract it should cease.

5.4 Number of anticipated bats

Investigators should balance maximizing capture success with minimizing risk to animal welfare if more bats are caught than can be safely and quickly handled. It can be extremely difficult to predict how many bats may be captured at any given time, especially for the first site visit. In general, bat activity and captures on a given night vary with geographic region, climate, season, weather, and habitat (discussed below). Examining peer-reviewed and grey literature for an investigator's target species and region can be helpful. Emergence counts may be helpful prior to capturing at roosts. Where possible, acoustic monitoring can provide a rough sense of activity in an area to help anticipate capture success of free-flying bats.

5.4.1 Geographic region/Climate

Generally, there are fewer bat species and fewer individual bats at more northern latitudes and at higher altitudes as average night-time summer temperatures are often sub-optimal for bats in these locations (Alves *et al.,* 2018). There are also fewer individuals in regions with endemic WNS, such as eastern Canada, because of severe population declines (ECCC, 2018). This has resulted in many of the affected species being listed as Endangered under SARA (ECCC, 2018).

5.4.2 Weather/Season

Bat activity in any region depends on local weather and season. Bats are typically less active at temperatures below 10°C (except perhaps at more northern latitudes and in higher elevation areas), in areas with high wind speeds, and when it rains (Erickson and West, 2002; Gorman *et al.,* 2021; Wolcott and Vulinec, 2012). It is not recommended to catch bats in the rain due to the increased energy demands when bats become wet (Voigt *et al.,* 2011). Additionally, wind can cause nets to billow, which bats can detect more easily (USFWS, 2007) and can also place bats caught in nets at greater risk of injury due to increased tension created by the stretched net strands on limbs and digits. As such, nets should be closed if billowing is evident. Due to ambient temperature and dietary requirements, bats in Canada are most active in the summer, and either hibernate through the late fall, winter, and early spring or migrate south. The exact timing of hibernation and migration for a given location depends largely on its temporal climate and thus is somewhat variable from year to year. Investigators should therefore consult local experts and weather records to establish the approximate timing of hibernation and migration.

5.4.3 Habitat

Bat activity also depends on habitat-type within a given season. 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 higher over ponds and other calm bodies of water, including puddles, because bats drink water upon emergence from their roosts (Ancillotto *et al.,* 2019; Broders *et al.,* 2003). Also, the adult stages of many species of insects that form the diet of Canadian bats emerge from, and swarm over, bodies of water (Clare *et al.,* 2011). Hibernacula and surrounding areas may be hot spots for bat activity because some bat species aggregate at them (i.e., swarm) to mate prior to the onset of hibernation (Randall and Broders, 2014). Similarly, roosts and their surrounding areas will likely yield much higher captures compared to captures of free-flying bats elsewhere.

6.0 Restraint, Handling, and Release

6.1 Bats in hand

Bats should not be held solely by wing tips, thumbs, or forearms as they will struggle and potentially damage flight muscles and break bones (Figure 9; Bat World Sanctuary). Different holds can be used depending on the desired outcome. For example, a common hold that allows easy transfer of a bat between personnel is to place the index finger between the scapula and use the thumb and middle finger to hold forearms at the sides (commonly referred to as 'Nelson' hold; Figure 10). Care must be taken, however, to ensure forearms are not overextended behind the back, which is speculated to strain wing muscles (Figure 10).

Figure 9. Bat held incorrectly by its fingertips and without proper PPE (Florent Valetti)

Alternatively, bats can be held by gently placing 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 grasping the bat's body in the palm (Palm grasp or hold; Figure 11). The thumb (or index finger) can be gently pressed on the bat's head to keep the bat's jaw closed to prevent biting. Using this hold, it is easy to expose anatomical areas for inspection, measuring, and sampling by carefully adjusting the grip to expose the desired body part (Figure 11). The bat can then be manipulated and measured with the dominant hand. For added security, the bat can be wrapped in a bat bag while grasped in the palm (Figure 12).

Figure 10. Left - 'Nelson' hold with wings at sides (Jared Hobbs). Right - Bat held with arms over-extended behind back (Adapted from: https://www.batcon.org/press/scholars-expand-research-capabilities-for-global-bat-conservation-2/).

Figure 11. Palm grasp (top - Jordi Segers) with wing open for examination (bottom left - Bob Brett) or being measured (bottom right - Jason Headley).

Figure 12. Holding a bat with a bat bag while processing for illustrative purposes only (Krista Patriquin). Note - it is NOT recommended to handle bats without gloves.

6.2 Holding bags and bins

Bats held for processing can be placed in a breathable fabric "bat bag" with a drawstring (e.g., 20 x 30 cm cotton drawstring bags) (Vonhof, 2006). Bags can be purchased from various suppliers (Appendix 1) or are easily made. Drawstrings provide a tight close and secondarily tying the drawstring tightly around the cinched top is the best method to prevent bats from escaping (Figure 13). Lastly, a clothes pin placed beneath the cinched opening is a further technique to ensure bats will not escape (Figure 13). However, while securing the bags with these methods, make sure the bats within the bag are not accidentally cinched, tied up or crushed by the clothes pin. Bags with frayed seams or loose threads should not be used (or they should be turned inside out prior to use) to prevent the bat's feet or thumbs from becoming entangled. Cloth bags are recommended for crevice-roosting bats (i.e., most Canadian bat species) that are accustomed to roosting in sheltered, confined spaces (Figure 13). Fine-mesh 'produce' bags (Figure 13) are recommended for *L. cinereus* because they exhibit less stress compared to when they are held in cloth bags (E. Baerwald and C. Lausen, pers. comm. 2022). Although disposable paper bags have been used to minimize transmission of pathogens and parasites, it is possible that bats held in them may experience heightened stress because they are unable to cling to the smooth paper. Bats can also quickly chew holes in paper bags, increasing the risk of their escape. Paper bags lined with plastic mesh may provide a suitable option. Bags made of airtight, non-breathable material (e.g., plastic bags) should not be used to hold live bats to prevent smothering.

Figure 13. Bags for holding bats with drawstring tied around cinched top. Top left - cloth bag (Krista Patriquin); top right - mesh bat bag (Cori Lausen). A clothespin (bottom - Jordi Segers) may be added to prevent escape (blue plastic clothespin) and used to label bags (wood numbered clothespin).

Larger holding devices (e.g., the Myers bag, nylon-net attached to a metal or plastic cylinder, minnow buckets, modified trash containers, and modified Styrofoam containers) have been used for holding groups of bats (Kunz and Kurta 1998). However, it is currently recommended that only one bat is placed within each holding device (see 6.3 "Number of bats per bag/bin") to prevent bat to bat transmission of pathogens and parasites.

Figure 14. Holding bin with bags hung on rod and heat pack in a sock on bottom (Jordi Segers).

To facilitate record keeping and management of holding times, bat bags should have unique identifiers, such as numbers, patterns, colours, or letters to track number of bats, capture times, species, and demographic data (if these can be assessed quickly at time of removal). Bats in bags should be carefully tied or hung a short distance from the capture site, preferably away from light, noise, and potential predators (e.g., mice, owls, other raptors). Bags can be hung off the ground on nearby objects with carabiners or plastic clothes pins, or by looping the drawstrings around an object. They can also be looped to, or clipped onto, a spare bat net pole, wire, metal rod or PVC/metal pipe (i.e., a material that can be easily disinfected) which is laid across a raised surface such as a processing bench (provided the bags are suspended and not at risk of being crushed by the activities of personnel), placed through predrilled holes in a large plastic bin and covered by a lid with holes to allow air flow (Figure 14) or tied between two trees. A warm water bottle or heat pack can be placed in a thick wool sock at the bottom of the bin to provide warmth, preventing torpor and/or facilitating rewarming prior to release. There should be sufficient space in the bin to ensure there is no possibility for direct contact between the bat bags and heat source, thus preventing overheating or burning the captured individuals. If a water bottle or heat pack is not available to rewarm torpid bats, affected individuals can be held in appropriately gloved, cupped hands for rewarming. If this does not work, as a last resort, bats in bags can be carefully placed inside an investigator's jacket or shirt, taking great care to not crush bats and ensuring there is no possibility the bat's claws or teeth can be in direct contact with the investigator's skin. It is not recommended to leave bats unattended in enclosed spaces (e.g., vehicles, rooms), where they might be lost, get injured, or bite a person should they escape their holding bag.

After bats have been released (see 6.7 "Releasing bats"), the bags should be turned inside out, shaken to remove any guano or parasites, and decontaminated before reuse (see 4.3 "Decontamination").

6.3 Number of bats per bag/bin

Multiple species should never be in the same bag, and ideally only one bat should be placed in each bag or bin to prevent the spread of pathogens and parasites between bats, as well as to minimize stress and fighting injuries (Edwards *et al.,* 2022). A female caught with one (or more) attached pup(s) should be released immediately. However, if data specific to breeding females and/or young are needed, place female and pup(s) in a bag together. Ideally, bags should not be reused on the same night. However, if the need arises, bags to be reused should be turned inside out and shaken to remove guano and parasites, should not be urine-soaked, and should only be used for individuals caught from the same colony.

6.4 Restraining devices

For taking measurements (e.g., wing morphometrics), applying devices (e.g., radio-tags, PIT-tags), and obtaining samples (e.g., biopsy punches), a restraining device can be very useful. The McMaster bat restrainer (Figure 15) has proven very effective for handling *E. fuscus* and larger species and can easily be modified for smaller species (Ceballos-Vasquez *et al.,* 2014). The advantage of this device is it allows a single person to restrain and conduct procedures on a bat, whereas two people are often required without the device.

6.5 Handling duration

6.5.1 General guidelines

Generally, bats should be held for the minimal amount of time required to collect data and released immediately thereafter. The Three Rs (see 3.0 "General considerations") can help investigators determine appropriate handling and holding durations. Depending on the projects' objectives, to *reduce* the number of individuals:

(1) If it is possible to identify recaptured individuals in traps (check for PIT-tag, bands, hair clippings, biopsy marks, or other identifiers; see 7.0 "Marking"), they should be removed first. If recaptured on the same night, general health can be quickly assessed (e.g., ensure no injuries have resulted from prior handling) and bats released. If recaptured within the same season, general assessment of health (e.g., mass), development (e.g., juvenile development), and reproductive stage can be quickly assessed prior to the bat's release. If recaptured in subsequent years, then obtaining the full protocol of measures (e.g., biometrics, demographics, biological samples) may be warranted.

(2) Balance cumulative stress of a colony with individual stress. In some cases, prolonged holding of an individual to meet the requirements of a protocol may be warranted to offset the cumulative stress if multiple recaptures or subsequent visits are needed to obtain the necessary data.

Below are general suggestions to help guide handling decisions.

It is recommended that bats be held in their bag for at least 10 minutes prior to processing to reduce stress. Normally, it is best to limit holding time to 4 hours, including time in net/trap (approximated based on frequency of net-checks and removal time), acclimation in holding bags, wait time for processing, and processing time. When held longer than 2–3 hours, consider providing bats with water and food prior to their release (see 6.6 "Provisioning bats"). Maximum holding time may vary with time of night, body condition, local environmental conditions, season, species, sex, age, reproductive condition, and torpor (see below), as well as permitted research activities and objectives. For example, Edwards *et al.,* (2022) recommends *M. lucifugus* be released within 30 minutes of capture as they experience elevated stress if held for prolonged periods.

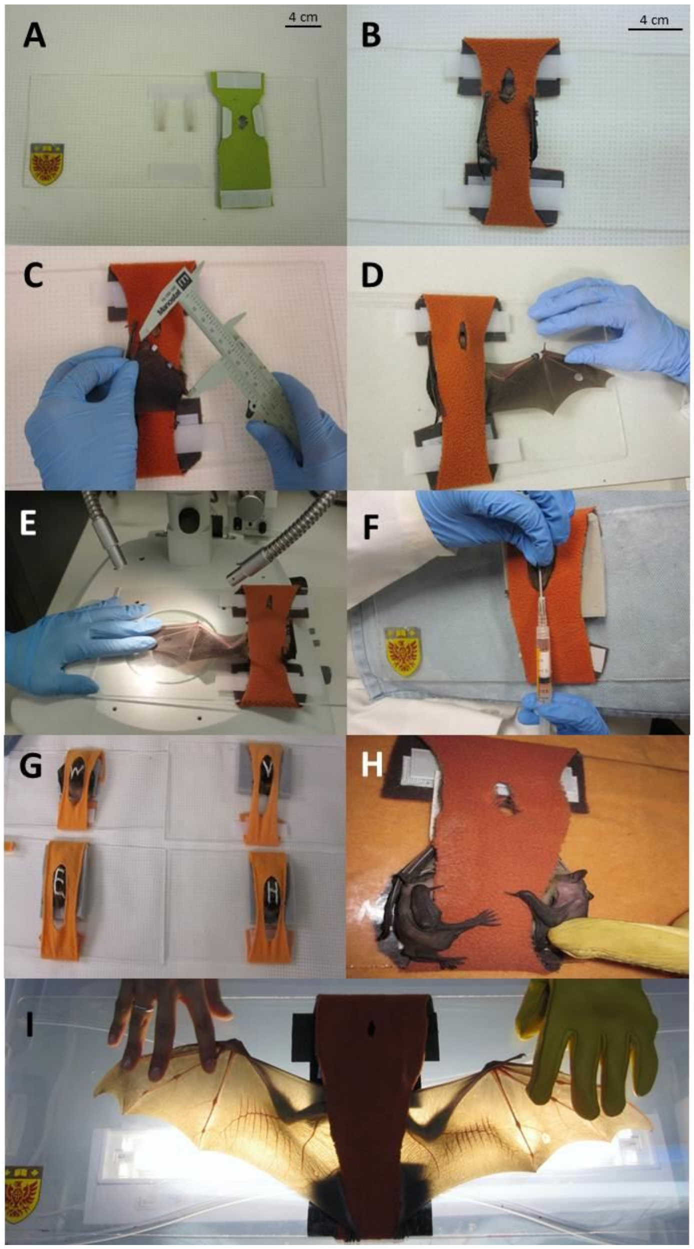


Figure 15. McMaster bat restrainer (provided by Paul Faure).

The experience of personnel can significantly influence handling time and must therefore be considered. Personnel should also be aware that captured bats will need to be managed through a 'triage' approach, where bats are prioritized based on risk of morbidity or mortality due to prolonged capture and handling (Table 1). In addition to the recommendations covered in this document, using previously successful protocols and working with experienced bat practitioners will help inform which bats should be prioritized.

6.5.2 Time of night

Within first hour of sunset: Normally it is best to hold bats for no more than 2 hours as they need to feed and drink after roosting all day. Food and water should be provided if bats must be held longer (up to a maximum of 4 hours).

Within one hour of sunrise: Bats still being held should be released immediately so they can return to their roost without potential exposure to diurnal predators. If time allows, consider providing food and water prior to release.

Anytime between sunset and sunrise: As above under 6.5.1 "General guidelines", but see below for other considerations.

It is important to carefully consider the length of the night when deciding how long to hold bats. As mentioned above, sufficient time must be available to carefully process bats and release them prior to sunrise. However, this will vary across seasons, as well as geographic location because night length can be significantly shorter at more northerly latitudes in the summer.

6.5.3 Local environmental conditions

As discussed above, captures of free-flying bats typically do not take place on nights with inclement weather that may reduce capture success. However, environmental conditions in some areas can change quickly. Personnel should be mindful of local weather forecasts and patterns and should be prepared for inclement weather. If temperatures drop below 10°C, bats should be released immediately as these weather conditions increase energetic demands of thermoregulation (Voigt *et al.* 2011). Obvious exceptions to these suggestions include work conducted in the winter (Slough and Jung 2008; Klüg-Baerwald *et al.* 2016), as well as at higher latitudes and altitudes characterized by colder nights where bats may remain active below 10°C (Patriquin and Barclay 2003, Wolbert *et al.* 2014, Slough and Jung 2008). Similarly, bats should be released at the onset of rain as it can also interfere with thermoregulation, as well as navigation (Voigt *et al.* 2011). That said, depending on the forecast, it may be advisable to hold bats in a dry, warm area until rain subsides rather than releasing bats in heavy rain. Similarly, it may be best to hold any bats that may have gotten wet in nets to allow them to rewarm prior to release. Additionally, wind speeds should be carefully monitored to mitigate potential injury to bats entangled in billowing nets. If entangling of bats occurs, those bats involved in a lengthy, difficult removal should be released immediately or as soon as possible if stressed and/or exhausted.

6.5.4 Season

Generally, night-time temperatures are lower and night length is shorter in spring and fall compared to summer, increasing the possibility that bats will enter torpor. In addition, a bat's energy budget is constrained during these periods as they attempt to recoup reserves lost during hibernation or build reserves to enter hibernation (Hranac *et al.* 2021). Handling and holding times should therefore take this into consideration to minimize additional stress on a bat's metabolic needs by reducing handling times (Table 1), keeping bats warm (but see 6.5.7 "Torpor"), and provisioning bats.

Capturing and handling bats in the winter is generally not recommended, unless investigators have experience working during this critically sensitive period (or will be working someone with experience). Disturbing bats during hibernation can be very costly because the metabolic rate required by bats to maintain normothermia at typical hibernaculum temperatures may be up to 400 times greater than that necessary during torpor (Thomas *et al.,* 1990). Moreover, free-flying bats caught during the winter do not readily go into torpor despite cold ambient conditions (C. Lausen pers. comm.). Capturing and handling bats at this time therefore puts them at considerable risk of over expenditure of much needed energy reserves. If investigators plan to capture and handle bats in the winter, they should consult with local experts and the literature. Holding and handling times should be kept to an absolute minimum (<1hour) to reduce negative impact on a bat's hibernation energy budget. Bats should also be provided extra warmth using a hot water bottle or heating pad (see 6.2 "Holding bags and bins") and should be provisioned with water and food.

In the event a bat is found outside hibernaculum in the winter, personnel are encouraged to consult their permitting agency or the federal/provincial/territorial agency responsible for the species, especially if it is a listed species. There are myriad factors that might result in bats emerging in the winter that should be considered when deciding the best course of action, such as ambient temperature, whether it is early or late winter, body condition, signs of poor health, proximity to known hibernacula, etc. Practitioners working in the winter are encouraged to consider these so they can prepare in advance. In some instances, euthanasia may be the most humane course of action in which case bats should be submitted for necropsy to determine if health issues may have led to emergence and/or may be affecting the population. However, in some cases, releasing bats may be the best option, particularly if practitioners do not have the authority or confidence to euthanize a bat; incidents should none the less be reported to the appropriate authority for further investigation and follow up if needed.

6.5.5 Species

Non-target species: Collect basic information (species, sex, and reproductive condition; band or PIT-tag information if present) and release. BUT see below for additional conditions.

M. lucifugus, M. septentrionalis, P. subflavus: may require special handling permits because they are listed as Endangered under SARA in Canada. This varies by region, depending on provincial and territorial Species at Risk legislation.

C. townsendii: may benefit from an acclimation period of ~15–30 minutes in a holding bag to minimize stress prior to further handling (C. Lausen, pers. comm.).

L. borealis and L. cinereus: these bats typically roost in foliage rather than in enclosed spaces and therefore mesh bags suspended in a quiet place are likely less (RISC, 2022). They may also benefit from a 15–30-minute acclimation period prior to further handling (C. Lausen, E. Baerwald, pers. comm.). These measures may be particularly helpful when handling males during periods coinciding with spermatogenesis (L. Bishop-Boros, pers. comm.). These species have been assessed by COSEWIC as Endangered (2022) thus further recommendations and permits may be forthcoming.

L. noctivagans: although no known special handling considerations exist for this species at this time, it has been assessed by COSEWIC as Endangered (2022) and therefore recommendations and permits may be forthcoming.

6.5.6 Morphometric and demographic data collection

Morphometric measurements and demographic data, such as sex, reproductive status, and age, are often useful for species identification and assessing population health. However, obtaining this information requires additional handling and therefore adds to the captured individual's stress. As such, investigators must carefully weigh the importance of obtaining each additional measure against increased handling time to ensure they do not exceed the recommended thresholds outlined in Table 1.

6.5.6.1 Body Mass

Where an accurate mass is needed, it is recommended to fast bats for one hour before weighing them to allow for elimination of recently consumed insects. The need to obtain an accurate fasted mass must be balanced against the potential risks of holding bats for the required time (see 6.5 "Handling duration"). Fasting is likely not necessary for most censuses and is not necessary when bats are captured at or near the time of emergence, as they are likely already fasted having not eaten since the previous night.

Figure 16. Bat wrapped in nylon in hand (left - Krista Patriquin) and on scale (right - Brock Fenton).

A spring scale (with 1g increments or less) or digital balance (with 0.1g increments) can be used to weigh bats in holding bags. Because it can be difficult to obtain a stable reading when bats are moving, it may be helpful to wrap an extra bag or excess bag material around the bat to immobilize it. Bats can also be temporarily restrained by wrapping them in knee-high nylons like a "burrito" (Figure 16). Alternatively, bats can be temporarily immobilized by placing them on the scale under a 'mesh' cup (e.g., pen holder) or paper cup. In all instances, remember to account for the mass of the holding bag or restraining material (weigh it and subtract from total weight, or tare it on the scale) when recording the bat's mass. Also, all materials should be disinfected between weighing bats (see 4.3 "Decontamination").

6.5.6.2 Forearm length

Forearm length is the standard morphometric measurement used to indicate the overall size of a bat, which is often used to differentiate closely related congeners. This can also be used in conjunction with body mass to determine body condition index in some species, although this may no longer be a recommended practice as body mass alone has been demonstrated as the best predictor of fat mass in insectivorous bats (McGuire *et.al.,* 2018). Forearm length is typically measured using calipers to measure from the base of the thumb to the elbow (i.e., entire length of the radius and ulna; see Figure 15, box C) to the nearest 0.1–0.5 mm (Quebec Ministry of Forests, Wildlife and Parks, 2021; RISC, 2021; Vonhof, 2006). It is important to be mindful of the amount of pressure used when closing the calipers to avoid potential damage to the wing. Some protocols recommend measuring forearm length multiple times and recording the average of all measurements as the most accurate technique (Vonhof, 2006).

6.5.6.3 Additional morphometric measurements

The collection of additional morphometric data may be essential in areas with species that are difficult to, or cannot be, distinguished visually and may even require additional identification techniques for accurate species identification (e.g., acoustic recording, genetics; (RISC, 2021)). Additional morphometric measurements may include tragus length, length of ear, total length, length of tail, and length of foot (RISC, 2021; Vonhof, 2006). Naughton *et al.* (2012). Species-specific literature should be consulted to determine which measurements are necessary based on the species diversity in your region, as well as the exact technique needed to accurately obtain the measurements. As always, investigators must be constantly aware of the additional handling time required for taking each measurement and guard against increasing the holding time above the project's predetermined threshold to obtain them.

6.5.6.4 Sexing

Males and females can be easily distinguished by gently examining external genitalia to identify the penis or vulva (Racey, 2009).

6.5.6.5 Reproductive status

Here we focus on the reproductive status of females. While male reproductive status may provide additional information about population health, establishing the reproductive stage of males can be difficult and time consuming (K. Patriquin, pers. obs.). Investigators interested in assessing male reproductive condition should consult Racey (2009), but should also be aware of the significant time commitment required to obtain this information.

Unless specified otherwise, the following descriptions of female reproductive stages are derived from Racey (2009).

Females with attached pups: Collect basic information (species) and release immediately.

Late-stage pregnant females (Figure 17a): Collect basic information and release immediately to preclude parturition of pups during handling and/or holding. Late-stage pregnant females can be identified based on: (1) large, distended abdomen laterally and ventrally, where underlying skin is visible through fur; (2) increased mass that can be 33% greater than normal expected range; (3) markedly, enlarged and distended uterus and/or skull of the fetus can be readily felt by gently palpating the abdomen; and (4) potential expression of milk when nipples are gently palpated. If a female does give birth during holding, place the holding bag containing mother and pup(s) in a quiet, secure place and allow them to bond (e.g., grooming by mother). Ensure the pup(s) is(are) latched to teat(s) before releasing. (see 6.7 "Releasing bats" for more details).

Early-stage pregnant females: Hold for a maximum of 1–4 hours, depending on time of night and body condition (Table 1). Consider provisioning with food and water if held for more than 2–3 hours (see 6.6 "Provisioning bats"). Early-stage pregnant females can be differentiated from late-stage pregnant females based on: (1) abdomen may be distended laterally, but less so ventrally and skin is not yet visible through fur; (2) mass may not differ greatly from normal expected range, but can be approximately 15% greater (Kunz *et al*. 1995); (3) mildly to moderately, enlarged and distended uterus can possibly be felt by gently palpating the abdomen; and (4) milk not likely to be expressed when nipples gently palpated.

Nursing females (Figure 17b): Hold for a maximum of 1–2 hours (Table 1) as they must return to their roost to nurse young. Nursing females can be identified based on worn fur or bare patches of skin around nipples, flaky (i.e., keratinized) nipple skin, and expression of milk when nipples are gently palpated.

Not obviously pregnant/non-reproductive females: Hold for a maximum of 1–4 hours, depending on time of night and body condition. Provision with food and water prior to release if held longer than 2 hours.

Post-lactating females (Figure 17c)/males (Figure 18): Hold for a maximum of four hours. Provision with food and water if held longer than two hours, and each hour thereafter. Post-lactating females can be differentiated from nursing females based on evidence of some regrowth of fur around nipples and milk can no longer be expressed from nipples.

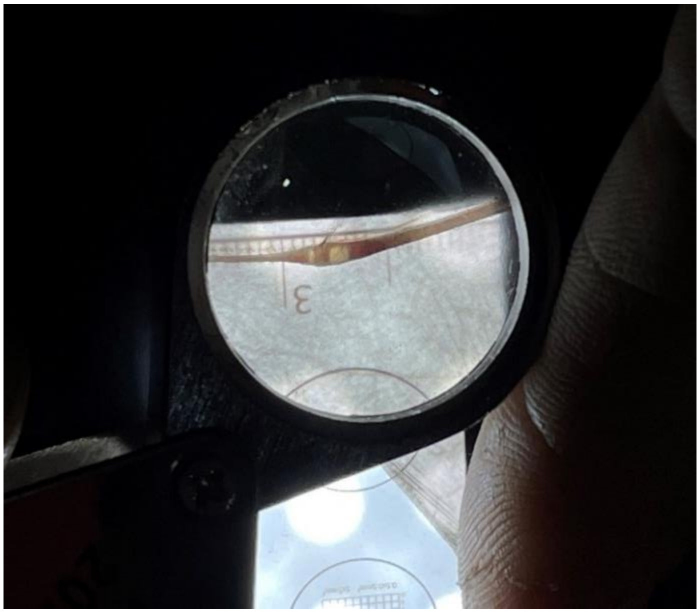
** In cases where many bats are captured in a short period of time, it may not be possible to establish demographic status while removing them from traps, resulting in the possibility that some bats are held longer than the suggested timeframes. Therefore, it is strongly encouraged to assess reproductive stage of bats within an hour of capture so those needing more immediate attention can be processed in a timely manner, for example: late-stage pregnant females, nursing females, and volant juveniles (see 6.5.6.6 "Age class").

Figure 17. Comparison of female reproductive stages: late-stage pregnant (top left – Jared Hobbs), nursing (top right – Jared Hobbs), and post-lactating (bottom – Brock Fenton).

Figure 18. Male bat (Jared Hobbs).

6.5.6.6 Age class

Volant juveniles: Volant juveniles can be differentiated from adults based on the presence of cartilaginous metacarpal and phalangeal epiphyseal growth plates (also known as "epiphyseal gaps") and tapered finger joints instead of having the 'knobby' appearance of adult finger joints once they are ossified after growth is complete (Figure 19; Brunet-Rossini and Wilkinson 2009). The metacarpal and phalangeal epiphyseal growth plates can be detected by shining a light through the finger joints and detecting translucent areas with increased light shining through them. Differentiating older volant juveniles from adults based on the presence of epiphyseal gaps can be challenging and time consuming. Examining tooth wear is often a reliable method for differentiating older volant juveniles from adults (see below). Current practice is to treat volant juveniles similar to that described above for non-reproductive females and adult males. However, it may be beneficial to prioritize them for processing as they may not be proficient fliers. (Buchler 1980).



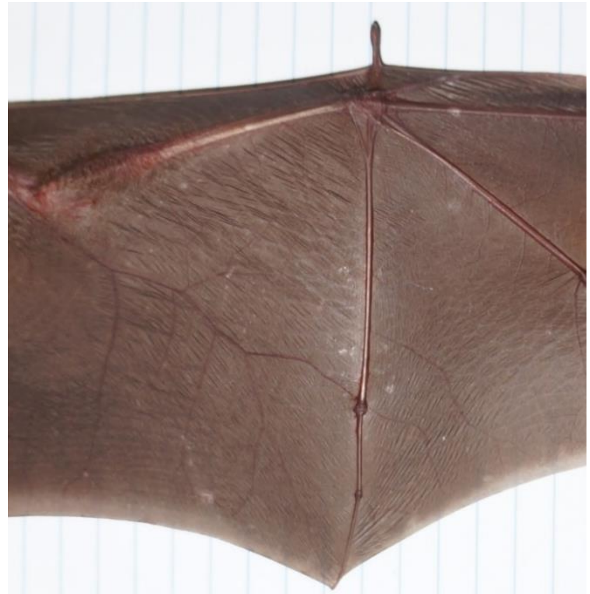


Figure 19. Metacarpals of juvenile bat showing epiphyseal gap (left – Hildegard Gerhach) and adult showing 'knobby' metacarpal joints (right – Krista Patriquin).

As bats age, canine teeth wear which can then be used as a relative index of age (Brunet-Rossinni and Wilkinson, 2009). The standard tooth wear scale contains 7 or 8 classes of relative wear, but wear varies with diet, which differs across species, as well as regions due to variation in prey availability and other factors (Christian, 1956, Holroyd 1993). For instance, wear will be faster and more pronounced on bats that eat harder-bodied insects (e.g., beetles) compared to those that eat softer-bodied insects (e.g., moths, dipterans) (Brunet-Rossinni and Wilkinson, 2009). Nevertheless, tooth wear can provide a relative index of age within a population to track bat life history. If time permits, investigators may therefore consider using a coarse 4-point scale to assess relative age using the upper canine teeth, where the first class applies to young-of-the-year and all other classes apply to adults (Figure 20; Figure 21):

1. Sharp canines
2. Slightly flattened canines
3. Obviously flat or angled with most of the canine tooth remaining
4. 1/2 or less of the canine tooth remaining

A 10X magnifying loupe is recommended for observing the upper canines, which are small (Figure 22). The easiest and most effective way to do this is to hold the bat in the palm of the non-dominant hand with the index finger under its chin and the thumb behind the head. To expose the teeth, reach up to the top of the head with the thumb and gently pull back (ie. slide the thumb from the top of the head to the base, and in doing so, this pulls the bat's head backwards and it opens its mouth). Rotate your wrist so that the bat's head is in front of your eyes (viewing through loupe). The bat's head is upside down making the view of its upper canines clear (Figure 22). Importantly, the tooth wear should be relatively similar bilaterally because if one canine tooth is much shorter than the other, it could simply be the result of a traumatic fracture rather than wearing over time.

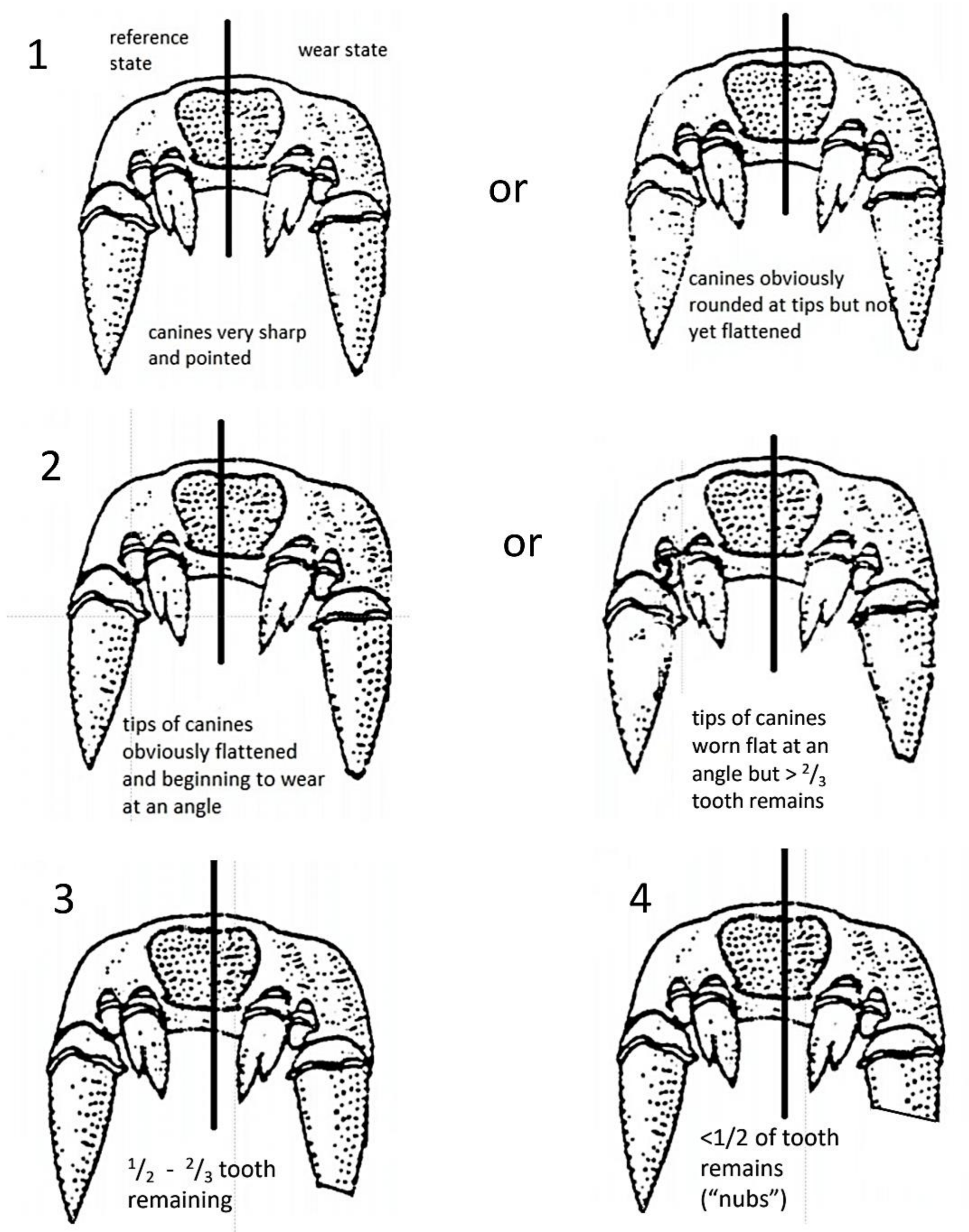


Figure 20. Schematic illustrating tooth class based on canine tooth wear used for coarse-level age estimation (Adapted from Holroyd 1993).

Figure 21. Tooth class 1 (Top – Jason Headley), Tooth class 3 (Bottom left – Krista Patriquin), Tooth class 4 (Bottom – right – Krista Patriquin).

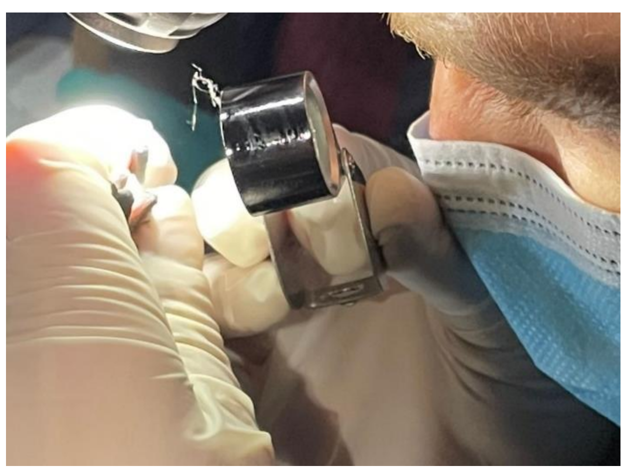


Figure 22. Examining teeth with loupe (Cori Lausen).

6.5.7 Torpor

Torpor is a thermoregulatory strategy used by bats to save energy and is accomplished by decreasing their internal body temperature to match that of lower ambient temperatures, significantly reducing their basal metabolic rate. Like all mammals, bats must maintain a body temperature to support the metabolic rate required for essential physiological processes which are optimized within a range of body temperatures known as the thermoneutral zone (TNZ). The TNZ for most Canadian bats is between ~27ºC and 37ºC, and they must use energy while at rest when ambient temperatures fall below the lower threshold of the TNZ, with more energy required the colder the ambient temperatures becomes (Stones and Wiebers, 1965; Studier, 1981; Cryan and Wolf, 2003; Willies et al. 2005). At temperatures lower than the TNZ, bats may opt to conserve energy by entering torpor. The frequency and depth of torpor varies with size and reproductive stage, as well as context (Neubaum, 2018). Smaller bats (e.g., *M. ciliolabrum*, *M.californicus*, *M. leibii*), for example, have a much higher TNZ and therefore go torpid quickly as temperature falls. By contrast, pregnant and nursing females tend to limit the frequency and depth of torpor as it inhibits fetal development and milk production (Audet and Fenton, 1988; Besler and Broders, 2019). Irrespective of size, free-flying bats captured in the winter limit the use of torpor and should therefore be kept warm to minimize the increased metabolic costs of thermoregulation (C. Lausen, pers. comm.).

Torpid bats are easier to handle, but arousal from torpor is energetically expensive, particularly in cold ambient conditions. Investigators must therefore carefully consider what is best for an individual bat's welfare with respect to potential energetic costs of remaining active while in holding versus the costs of rewarming prior to release if bats go torpid. Investigators should also consider the implications for collecting data. For example, it is much more difficult to obtain blood from a torpid bat and/or cold bats (e.g., *M. lucifugus* at temperatures < 10ºC – T. McBurney pers. comm.). Also, bats recently aroused from torpor may not produce typical echolocation calls so any calls collected to serve as reference calls for future species IDs may not be representative of the species. As described above, bats can be kept warm, or rewarmed (~15–20 minutes to rewarm), using exogenous heat sources (see 6.2 "Holding bags and bins"). If possible, bats aroused from torpor should be provided with water and food prior to their release (see 6.6 "Provisioning bats").

6.5.8 Poor health

Bats in poor health should be minimally handled, have no contact with other captured individuals, and should be released as soon as possible. Clinical signs of poor health include:

1. Emaciation, where the body mass is <60% of reported maximum body mass for the adults of the species in late August and early fall, recognizing that juveniles can weigh less than their adult counterparts. There are also sex differences between males and females, and the body mass of bats fluctuates significantly over the calendar year based on metabolism of their body energy stores correlated with their annual life cycle, including depletion and accumulation at various times associated with the summer, swarming, migration, and hibernation periods (Jonasson and Guglielmo 2016; Gallant and Broders 2015; Lacki *et al.* 2015; Jonasson and Willis 2011; Kunz *et al.* 1998).
2. Dehydration, where there are the following variable clinical signs depending on the degree of dehydration: dry and tacky mucous membranes; stringy, mucus; dull, dry, and wrinkled flight membranes; sunken eyes; and skin that stays tented when pinched (Lollar 2018).
3. Listlessness, where the eyes may be open, but bats show little to no reaction to stimuli like touch and light (i.e., do not open mouths, do not attempt to get away, do not vocalize), and

body is limp when held. Although some of these signs may be confused with torpid bats (e.g., unresponsive), eyes are often closed while in torpor and the body is not limp when held. Also, overall responsiveness increases in torpid bats as they warm, when they may also make distinctive, audible vocalizations.

4. Abnormal behaviour, where bats can be extremely aggressive by biting and struggling; uncoordinated and unable to fly; and/or very passive and unresponsive. All these behaviours can be indicative of rabies (Constantine, 2009) so an animal with such clinical signs should be examined (See 10.0 "Euthanasia") by a wildlife health professional or veterinarian to determine if the bat should be euthanized and submitted for post-mortem examination and rabies testing. If this is not possible, the affected bat should be placed in a safe roosting site where human and domestic animal contact cannot occur and monitored for the next 24–72 hours. After this observation period, the bat should be submitted for post-mortem if it dies, or if clinical signs persist or worsen, leading to its euthanasia for humane reasons.

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. A torpid bat should become more alert when warmed.

6.6 Provisioning bats

Investigators should always carry water and food for provisioning bats. A sterile plastic eye dropper or syringe can be used to deliver water to bats orally. To do this, allow a single drop to touch the bat's mouth; normally bats respond immediately by licking the water. If a bat does not drink after being offered water twice it should be released instead of prolonging its holding time (Lollar, 2018). However, if a bat is assessed as severely dehydrated (see 6.5.8 "Poor health"), it should be released immediately.

The simplest and most nutritious option for feeding bats is to carry canned veterinary quality cat food, which can be diluted with water and delivered with a plastic eye dropper or syringe. Other sources of nutrition in the field include wet ferret food and high glucose veterinary supplements (e.g., Nutrical™), though the latter is not as nutritious. Mealworms are also commonly used to feed bats, but bats naïve to mealworms may not initially accept these. To facilitate this, it may help to remove the mealworm's head and squeeze the viscera from the carapace into/on the bat's mouth. Once the bat licks the viscera, it will often be willing to eat more at which point whole worms can be offered. However, whole mealworms can be difficult for smaller bats (e.g., < 5g) to chew and swallow and may therefore only accept viscera. Pupae and adults (beetles) are distasteful to bats and therefore only the larval 'worm' should be fed to bats (C. Lausen, pers. comm.). It is advisable to feed bats over a clean surface so it is easier to retrieve any dropped live mealworms and therefore prevent accidentally releasing them into the environment. When not in use, mealworms can be stored in a refrigerator or cooler to delay development into pupal and adult forms, but ensure they are not exposed to freezing temperatures which will kill them. Forceps can be used to mitigate the risk of being bitten while feeding bats, but ensure bats do not bite onto the forceps. To do this, hold mealworms in the very tip of the forceps with as much of the exposed mealworm's body as far away from the forcep tips as possible and place the exposed mealworm section near the bat's mouth.

As with all materials that have come into contact with individual bats, droppers, syringes, and forceps should be decontaminated between bats (see 4.3 "Decontamination").

6.7 Releasing bats

Bats should be released at, or near, their site of capture under favourable weather conditions (i.e., warm, dry, low winds). When releasing bats, if they are not obviously active (i.e., vocalizing, biting, squirming), ensure they can fly by doing a 'test flight' where bats are held near the base of the tail to encourage use of their wings (Figure 23). Bats that are ready to fly will flap their wings quickly and powerfully and can be released from hand at standing height, approximately 1.5 – 2m above the ground, by having personnel raise the arm holding the bat to head-level to ensure bats have sufficient ground clearance prior to release (Bowen, 2020; Haarsma, 2008). The release height will need to be higher for larger species, pregnant females, females carrying pups, and volant juveniles; this can be achieved by carefully standing on a raised surface, such as a vehicle, picnic table, ladder, or chair. In addition, when releasing a female with attached pup(s), it is best to avoid the tendency to place a hand under the pup for security. If a pup contacts the surface of your hand, prior to release, it may unlatch from the mother. Follow bats with a light to ensure they have flown away.

Figure 23. Test flight (left – Krista Patriquin; right – Brock Fenton).

If a bat does not successfully fly away, assess whether it was due to insufficient clearance by trying a second release higher off the ground. In this instance, bats should also be assessed for torpor and allowed to warm longer if necessary, prior to a second flight attempt. Occasionally the wing membranes get 'stuck' together if bats are held for prolonged periods, which can be remedied by gently opening the wings manually several times. To separate them, gently pull on the bat's forearm and index finger. In the event a healthy bat does not fly away, it can be placed high on a platform, ledge, tree trunk, or branch in an area where they can crawl to a higher spot or shelter, but away from clutter that may inhibit flight (Battersby 2017; Bowen 2020). This is not recommended near dawn as bats will be at risk of predation by crepuscular predators. If possible, bats should be monitored to ensure they have flown away or if further action or intervention is warranted (see 6.5.8 "Poor health").

7.0 Marking

Listed below are general suggestions for different types of marking that may be used for censuses.

7.1 Short-term marking techniques

7.1.1 Water-soluble marker

Water-soluble, non-toxic markers can be used to mark the hair of captured bats to track recaptures within nights. This technique may not be useful for tracking recaptures across nights as grooming could remove the mark. As with other equipment and materials, tips of markers should be disinfected (see 4.3 "Decontamination").

7.1.2 Non-toxic temporary hair dye

Non-toxic temporary hair dyes without bleach are available in many colours. This may allow short-term individual identification by using unique combinations and anatomical placements of colours.

7.1.3 Bee marking tags

Tiny coloured and numbered discs normally used to mark bees have also been used to temporarily mark individual bats (T. McBurney and M. Jones, pers. comm.). Tags can be glued to a bat's hair using surgical adhesive. How long these tags remain on bats is unknown.

7.1.4 Hair removal

Removal of small patches of hair could be used for tracking recaptures within a season at different locations or times. Patterns of hair removal may not work for individual identification of bats when captured in large numbers because the haired surface area of a bat limits the number of possible unique combinations. If possible, the removed hair can be kept for other studies (e.g., stable isotope or heavy metal analyses). Unless hair is being removed to attach radio-tags, it is advisable to avoid the scapular region and instead remove hair from near the tail where is more easily accessed. Also, the scapular region is more exposed when roosting and would therefore be more subject to heat loss if its hair has been removed. Hair on the ventral side is typically harder to access and is much shorter in length, increasing the possibility of accidentally cutting the skin. Hair should not be removed near wings due to the risk of accidentally lacerating wings. If hair will also be used for isotope analysis, investigators should be aware that up to 1cm^3 of hair may be needed and that the signatures may differ depending on the anatomical region of hair removal (RISC, 2022).

Figure 24. Scissors for removing hair. (e.g., http://www.roboz.com)

Use small, curved, blunt-tipped micro-dissecting scissors or cuticle scissors (Figure 24) to remove hair close to the skin, taking care not to cut the skin. Enough hair should be removed so bare skin is visible upon recapture; to check, brush remaining hair over the area to ensure the cut patch remains visible (Figure 25). Care should be taken to avoid removing too much hair as this could negatively influence thermoregulation (up to 1cm^3, the amount needed for isotope analysis). To minimize heat and water loss during hibernation, hair removal is not recommended between September 30 of a given year and May 31 of the following year.

Figure 25. Hair patch removed for marking, hair sample, and radio-tag attachment (Lori Phinney). Note - patch would be smaller if only for marking.

7.1.5 Wing or tail biopsy punches

If your project requires biopsy punches, the fresh or recently healed lesions from the punches can be used to identify recaptures within a season.

7.1.6 Light tags

Chemiluminescent tags, or light tags, can be used to track an individual after release, which can be useful for obtaining reference echolocation calls, or to observe flight and foraging behaviour (Barclay and Bell, 1988; Horvorka *et al.,* 1996). The benefits of this marking technique should be carefully weighed against potentially increasing the risk of predation, which remain unknown. However, at least one study revealed that light tags remain attached for up to 48 hours and continued to glow (Timofieieva *et al.,* 2019). Non-toxic miniature glow sticks (2.9 x 24mm) can be glued to the bat's fur using non-toxic glue sticks, such as those used for children's crafts (RISC, 1998, 2021). It is not necessary to clip hair for attaching light tags.

7.1.7 Radio-tags for locating roosts

To locate roosts for surveys, radio-tags can be attached to bats caught while commuting or foraging (i.e., not in or near roosts) to subsequently track those individuals back to their roost(s). The smallest radio-tag available should be applied to meet the research objective while ensuring the use of radio-tags does not compromise flight. To determine the maximum mass of a transmitter that can be safely used, the 5% "rule" is often applied, where the mass of the transmitter should be no more than 5% of a bat's fasted mass (Aldridge and Brigham, 1988; O'Mara *et al.,* 2014). How broadly the 5% rule should be applied remains uncertain as a bat's ability to carry a load depends on wing morphology relative to body size, which varies by species, sex, age, and reproductive status. Indeed, 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). Unless necessary to meet research objectives, investigators should avoid placing radio-tags on the following: (1) pregnant females because they are physiologically vulnerable; (2) juveniles captured early in the season (i.e., with large epiphyseal gap) because they are not yet proficient fliers; (3) smaller species like *M. ciliolabrum* because small enough tags are not available at the time of writing this document (RISC, 2021).

To attach a radio-tag:

- Activate the transmitter and ensure it is working properly.
- Place the bat in a restraining device, dorsal side up (Ceballos-Vasquez *et al.* 2014; Figure 15; Figure 26) with back exposed, or place the bat's ventrum on a soft but firm surface atop a table and hold the bat firmly with forearms tucked in at its sides. Placing fabric over the bat's head and allowing the bat's feet to grip to a surface may reduce stress.
- Locate the center of the back between the scapulae.
- Hair may be removed prior to attachment, depending on the project's objectives.
    - If radio-tags equipped with temperature sensors are used, a patch of hair the size of the transmitter must be removed to expose the skin's surface (see 7.1.4 "Hair removal") for an accurate temperature reading. If the skin is accidentally cut, do *not* attach a transmitter. Place a very thin layer of surgical adhesive on the wound, allow the glue to dry, and release the bat immediately.
    - If temperature is not being recorded, transmitters may remain better attached if hair is not removed (Brigham, n.d.). At the same time, this may lead to significant hair loss at, and around, the attachment site when the tag un-attaches (i.e., falls off).
- Using a small craft store paint brush, apply a very thin layer of surgical latex adhesive* (see note regarding adhesives below) between the scapulae (either directly on clipped or unclipped hair), as well as on the flat side of the transmitter.
- Allow glue to begin to bubble and become tacky before attaching the transmitter. Generally, this takes <5 minutes, but read manufacturer's instructions as drying times can be considerably less and may in fact dry faster than expected (Carter *et al.* 2009).
    - Note: the weight of the adhesive is also an additive factor in the 5% rule.
    - More glue is not better as it may cause the transmitter to loosen more quickly (Carter *et al.* 2009; Kunz, 1988).
- Place the transmitter on the bat, with antenna facing toward the tail while holding it in place for 1–5 minutes. Push adjacent hair on to any glue around the edges of the transmitter, or 'frost' the tips of adjacent hair with surgical adhesive and push it onto the glued edges of transmitter; this creates a more secure attachment. Allow the glue to dry fully before releasing the bat (~15–30 minutes; depends on humidity). A small amount of baby powder can also be applied (using the tip of a pencil eraser) to the glue after drying to prevent adhesion to foreign objects. Note: This entire step can be omitted if the goal is to locate the bat and remove the transmitter within a few days.
    - To prevent the bat from moving or attempting to remove the tag while the glue dries, the bat can be secured in a bat bag by wrapping excess fabric and drawstring

around the bat, while ensuring the antenna is not bent. This can be achieved by placing the bat headfirst in the bag, with the antenna facing the opening of the bag.

- Prior to release:
    - ensure the glue has dried by very gently attempting to lift the transmitter away from the bat's back.
    - it is also advisable to ensure the transmitter is still functioning and its frequency has not drifted.
- To remove tags, adhesive can be gently scraped from the fur and transmitter using a fingernail. If needed, a solvent (consult adhesive Material Safety Data Sheet (MSDS)) may be applied sparingly.

* Note: Many investigators have used Skin-Bond surgical adhesive, but this product is no longer available. Alternative adhesives include Vetbond Tissue Adhesive, Perma-Type Surgical Cement [which may also no longer be available, C. Lausen, pers. comm.], Torbot Bonding Cement, and Osto-bond (Carter *et al.* 2009). There also exists a brand in Europe: Sauer-HAUTKLEBER, type 50.01; Manfred Sauer GmbH (Timofieieva *et al.,* 2019). To prolong effectiveness, adhesives should be stored in the refrigerator when not in use. New bottles should be purchased each field season as the glue thickens over time, which reduces effectiveness (Kurta *et al.,* 2009). Alternatively, glue can be thinned with an appropriate solvent, usually hexane, that can be purchased from the same manufacturer (but see product's MSDS; Carter *et al.* 2009). Surgical adhesives containing methacrylate should be avoided due to their exothermic reactions and risk of inadvertently burning tissue. Some practitioners have used superglue to attach transmitters. While some recapture data indicate that there are no visible negative outcomes associated with this practice (T. Buchanan pers. comm.), it is generally discouraged. Surgical adhesives have undergone extensive testing to ensure animal safety, while superglue products have not. As such, any potential negative outcome is unknown.

Figure 26. Bat in restraining device with transmitter (Krista Patriquin).

7.2 Long-term marking techniques

7.2.1 Bat bands

Several commercial suppliers make bands that can be used for marking bats (Appendix I). Generally, bands of any type can damage wings if not applied properly, which should be carefully considered when choosing this method (Baker *et al.,* 2001; Lollar and Schmidt-French, 2002). Some census projects may warrant the use of bands as they can enhance long-term monitoring. For example, bands allow visual identification of previously captured bats without the need to recapture and handle the animal. Using unique combinations of colours can also allow quick identification of known individuals. Additionally, bands may be a preferred marking option if a census is taking place during fall swarming season when PIT-tagging is not recommended because associated wounds may not heal as readily at this time of year (see 7.2.2 "Passive Integrated Transponders").

Coloured, numbered, plastic split-ring bands designed for marking birds have been used for marking bats because they aid in visual identification of individuals. When choosing band size, a rough guide is to choose bands with an internal diameter equivalent to ~7% of a bat's forearm length*, with the smallest band height and largest gauge possible. This will ensure bands are not too large and thus prevent fingers bones from getting caught. It is best to modify plastic split-rings prior to application as they have sharp edges that can damage, or become embedded in, wing membranes (Lollar and Schmidt-French, 2002). To do this, clip the corners with a nail clipper and use a nail file to remove any sharp edges and widen the gap to fit more loosely around the forearm (Figure 27). Because bats can chew off plastic bands, long-term monitoring projects should consider pairing these bands with PIT-tags or using aluminum bands instead.

* Note: There is no source or empirical data that determined if using a band size diameter of approximately 7% of a bat's forearm length is appropriate. Future research could investigate appropriate band sizes using forearm length as an index. The guiding value of 7% is used in Western Canada and is sometimes rounded to find the appropriate band size (C. Lausen, pers. comm.). Several band sizes need to be on hand during banding due to variation of individual and species forearm length and diameter.

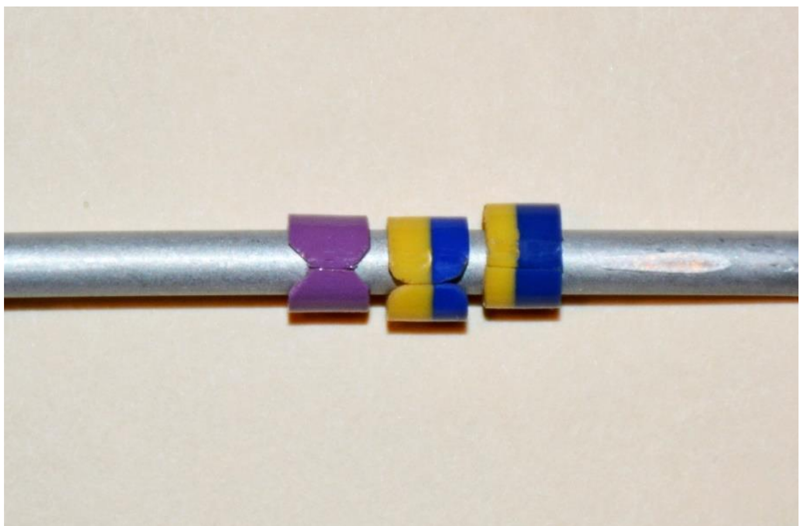


Figure 27. Modified split ring (Robert Barclay).

If individual identification of bats from a distance is not needed, aluminum lipped forearm bands may be a better choice because they are rounded with lipped edges that minimize the risk of wing damage (Figure 28). That said, aluminum lipped bands are also not without risk (Baker *et al.* 2001). The same guidelines for choosing plastic split ring size discussed above also applies to aluminum bands. Aluminum bands can also be numbered or lettered for individual identification of recaptured bats. Incoloy lipped bands that are more resistant to corrosion are also available from some suppliers. In addition, coloured, anodized lipped bands are also available, which could allow some level of individual identification without capture. When using metal bands, bat banding pliers are recommended to ensure the bands are not applied too tight. Circlip pliers are also recommended for removing bands (e.g., if band applied too tightly, recaptures). If bleeding occurs during band application or removal, it can be stopped by applying direct pressure or a hemostatic agent (Kunz and Weise, 2009).

Bands should be placed on the forearms (i.e., over the radius and ulna) of bats and should be sufficiently tight so they do not come off. At the same time, they should be appropriately loose so they can move freely along the length of the forearm without causing abrasions or tears (Kunz and Weise, 2009). To do this, place the band over the distal end of the forearm (i.e., by the thumb) and subsequently slide it partway towards the body on the forearm to the level of the distal attachment of the propatagium before closing it by gently applying pressure with one's fingers. After applying the band, fully extend and close the wing several times to ensure the bat's fingers and/or propatagium do not get caught in the band and the band cannot slide over, or entrap, the elbow joint. Inexperienced personnel are encouraged to practice applying and removing bands on a small tree branch or similar-sized object prior to attempting this procedure on a bat.

Sikes *et al.* (2016) suggest making a small incision in the wing membrane immediately below the bones of the forearm to insert the band through during application, presumably to prevent the band from moving too much along the forearm. This technique is generally discouraged for most bats in Canada because it is invasive and unnecessary. However, a small slit in the wing membrane may be useful to place the ring though when applying plastic split rings on *L. cinereus* and *L. borealis* because these bands can get caught on their propatagia, which are furred and larger compared to other Canadian bat species. However, long-term outcomes of this practice have not been formally documented and it should therefore be performed only by the most experienced practitioners, preferably with an approach to assess its safety. At the time of writing this report (March 2023), the effectiveness of lipped bands for lasiurine bat species is unknown and warrants investigation because there is increased interest in banding these species to establish their migratory routes through mark-recapture studies.

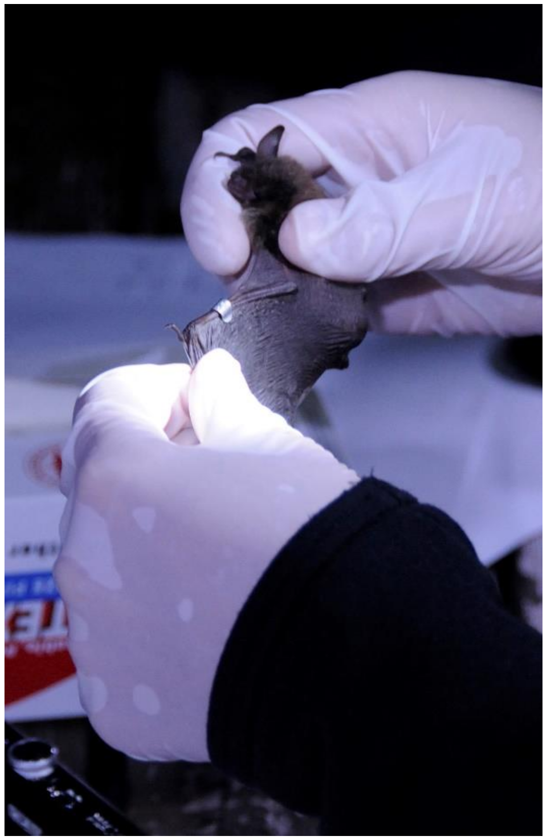


Figure 28. Lipped aluminum band on bat (Brock Fenton).

Regardless of band type, it is advisable to check band integrity prior to deployment in the field. For instance, band edges may not align properly when closed which could result in micro-tears on wing membranes (Ontario Ministry of Natural Resources and Forestry, OMNRF, pers. comm.). Similarly, pliers should also be tested and labeled prior to their use in the field to ensure the appropriate size that allows properly fitted bands that are not too tight when applied to the bat.

7.2.2 Passive Integrated Transponders

Radio frequency identification (RFID) tags, such as passive integrated transponder (PIT) tags and microchips, are commonly used to mark bats. PIT-tags are injected subcutaneously and have unique alphanumeric codes that can be detected and recorded using portable handheld readers or permanent readers installed at roost exits. Injecting PIT-tags requires additional personnel training and increases handling time and stress, as well as project costs. The use of PIT-tags is therefore discouraged for short-term identification studies in bats. Instead, PIT-tags are best suited for long-term population and behavioural studies where re-sightings are likely.

For studies requiring the use of PIT-tags, personnel should receive training on subcutaneous injections, which could include practice on objects like a chicken breast with skin. When injecting PIT-tags, one person can restrain the bat by hand while another person injects the tag. Alternatively, a restraining device (e.g., Ceballos-Vasquez *et al.,* 2014; Figure 15; see 6.4 "Restraining devices") can help limit a bat's movement and therefore reduce the risk of injury. To inject a PIT-tag:

- Removal of hair is not recommended as it could interfere with thermoregulation, increase handling time, and elevate stress. Instead, use a cotton swab to apply 70% isopropyl alcohol between the scapulae in a circular motion to part the hair outwards and expose the skin at the injection site (van Harten *et al.* 2020).
- Create a 'tent' of skin on the bat's back by pinching and lifting it bilateral to the two scapulae with thumb and index finger.
- Slowly insert the sterile needle containing the tag longitudinally, along the length of the body into the 'tent', ensuring the tip does not penetrate through the skin on any of the other sides of the tent.
- The needle should be relatively shallow and nearly parallel with the bat's body to ensure the needle remains underneath the skin tent and above the underlying muscle and spinal column.
- There will be an initial resistance as the needle enters the skin and then little or no resistance once the needle penetrates the subcutis.
- Carefully continue to insert the needle until the insertion mark on the needle shaft reaches the skin's surface.
- Slowly depress the plunger on the syringe and carefully watch to ensure the tag is inserted into the subcutis of the skin tent, remaining there and not exiting through the skin on the other side.
- Slowly withdraw the needle, ensuring the tag is fully inserted beneath the skin; massage the tag away from point of insertion into the subcutis to prevent the tag exiting through the injection hole.
- If the skin is accidentally punctured on the other side of the tented skin, seal the wound with surgical glue.
- Close the puncture site with surgical glue to prevent tag loss (van Harten *et al.* 2021).
- Allow bat to rest until glue has dried.

Other considerations when using PIT-tags:

- 12 mm tags appear to provide more reliable, long-term detection compared to 9 mm tags (Sandilands and Morningstar 2021). However, smaller tags may prove equally reliable (OMNRF, pers. comm.), particularly in cases where 12 mm tags may be too large for some species (e.g., *M. leibii*)
- Use extra care with juveniles, as their skin is not as loose as that of adults, or avoid technique altogether if a 'tent' of skin cannot be safely created.
- Use of surgical adhesive prevents tag loss, but also results in hair loss which should be taken into consideration (van Harten *et al.* 2019).
- Injecting PIT-tags is not recommended just prior to hibernation or during the winter because of the possibility of delayed healing during these periods, which can lead to an increased risk of secondary infections if these iatrogenic wounds remain open.

7.3 Methods not recommended

Several past marking methods are no longer recommended when censusing bats, including freeze marking, bleach, nail- and toe-clipping, ear-clipping, fur bleaching, tattooing, and the use of beaded necklaces (CCAC, 2003; Kunz and Weise, 2009; Sikes *et al.,* 2016). Specifically, freeze marking and fur bleaching may damage underlying tissues. The use of nail-, toe-, and ear-clipping are not encouraged because they are markedly invasive and can interfere with grooming, roosting, navigation, and foraging behaviours (Kunz and Weise 2009), while tattooing requires extensive training and can be time consuming. The use of bead necklaces may result in choking, abrasion, skin irritation, and increased predation (Jackson, 2003; Kunz and Weise, 2009; Losada *et al.,* 2021) These methods are therefore discouraged because they are unnecessarily invasive and pose a risk of harm to bats. Moreover, there are newer methods (described above) to safely mark individuals.

8.0 Biological Samples

Biological samples (e.g., blood, fluid, tissue, hair, guano, and ectoparasites) are often not required for bat census work. Investigators interested in these techniques can consult the sources provided here, as well as Brewer *et al.* (2021). More detailed guidelines are, however, provided for flight membrane biopsies and the collection of fecal samples as they are common to census work). Investigators should also note that federal/provincial/territorial, inter-provincial/inter-territorial, or international permits may be required to ship materials to appropriate laboratories for analysis.

8.1 Urine samples

- Bassett (2004)
- Kunz and Parsons (2009b)
- Pilosof and Herrera M (2010)
- Greville *et al.* (2022)

In addition to suggestions outlined in these sources, others have successfully obtained urine samples from bats before removing them from mist-nets. Because a captured bat often urinates when it is first touched, an investigator can hold a capillary tube over the genitals to collect any available urine prior to extracting the bat from the net. This technique may not be recommended for species that appear to display higher acute stress when initially captured (i.e., *L. cinereus* and *C. townsendii*) and thus may require an acclimation period of 30 minutes before handling for collecting samples.

8.2 Milk samples

- Kunz and Parsons (2009b)

8.3 Blood samples

- Kunz and Parsons (2009b)
- Eshar and Weinberg (2010)
- Hoffmann *et al.* (2010)
- Smith *et al.* (2010)
- Hooper and Amelon (2014)

* Note: It is much more difficult to obtain blood from a torpid bat and/or cold bats (e.g., *M. lucifugus* at temperatures <10ºC; T. McBurney, pers. comm.).

8.4 Biopsy punches

Biopsies from the wing or tail membranes of bats can be used for genetic analyses (e.g., species identification, relatedness) and occasionally for the diagnosis of WNS. As with other procedures, it is highly recommended that personnel receive appropriate training prior to obtaining biopsy punches from live bats, including practice on a non-animal model such as a lightly stretched surgical glove.

Wing membranes are easier to access (for a single person) and are less vascularized than tail membranes, thus reducing the risk of damaging major blood vessels (Hoffmann *et al.,* 2010). However, tail membranes heal faster and tissue samples from this location contain a higher concentration of DNA compared to samples of similar size from the wing membrane (Faure *et al.,* 2009). That said, Broders *et al.* (2013) reported an incident of *M. septentrionalis* becoming accidentally caught when a car antenna passed through the tail membrane at the biopsy site, as well as a record of a torn tail membrane at the biopsy site (Broders *et al.* 2013). The potential for further damage after release may be greatest for species that rely heavily on tail membranes for capturing prey, and therefore samples taken from wing membranes should be carefully considered instead.

Detailed instructions for obtaining flight membrane biopsies can be found in Vonhof (2006) and are briefly updated here. To obtain a sample, a bat's wing or tail membrane is stretched over a disinfected firm surface (e.g., cutting board, surface of McMaster restraining device) (see 4.3 "Decontamination"). A 2-mm punch tool is recommended for myotid bats, whereas a 3- or 4-mm punch may be used on larger species (RISC, 2021).

Place the biopsy punch in a location that avoids major blood vessels (which can be located by shining a light through the wing); some researchers prefer a location near the knee (Figure 29). Ensure the skin is taut so the resulting hole is the size of the punch and not larger (Figure 30). Press straight down on the tool firmly and rotate several times to ensure a clean cut.

Figure 29. Diagram illustrating 'magic triangle' (outlined in red) for biopsies (adapted from photo by Brock Fenton).

Figure 30. Bat tail (top – Jordi Segers) and wing (bottom – Krista Patriquin; taken before widespread use of gloves) stretched for biopsy.

Often the sample remains inside the lumen of the biopsy punch. If the sample is being stored in a vial containing a preservative solution, place the tip of the tool in the vial and gently tap on the opposite end of the tool until the sample is released. If the sample is not being stored in a preservative solution, a similar approach could be used by placing the tip of the tool in a small vial of water, subsequently retrieving the sample with sterile forceps. Alternatively, the sample can be retrieved from within the tool with sterile fine-tipped forceps while taking care to avoid pushing the sample farther into the lumen and up the shaft. Sterile forceps can also be used to remove the sample if it remains on the cutting surface. A new biopsy punch is strongly recommended between bats, especially if genetic work is being considered. However, if necessary, biopsy punches can be reused until they are dull and no longer make clean cuts. Used biopsy punches (if reused) and forceps should be sterilized between each individual bat to ensure DNA from bats previously sampled does not contaminate the current sample. Tools can be flame-sterilized, allowed to cool, immersed in ethanol, and allowed to dry (American Museum of Natural History, 2018). Alternatively, tools can be sterilized by placing them first in bleach, followed by water, then ethanol and allowed to air dry.

8.5 Hair

See 7.1.4 "Hair removal"

8.6 Fecal samples

Fecal samples are often easily obtained from within the holding bags of bats that have been kept for 30–60 minutes if they were captured at least one hour after emergence. Storage of samples depends on the project's objectives. For example, storage for DNA analysis differs from storage for diet analysis (Brewer *et al.,* 2021; USFWS, 2020)

8.7 Ectoparasites

Some studies require collection of ectoparasites. The best form of removal depends on the parasite species. For example, small wing (*Spinturnix americanus*) and ear mites (*Trombiculidae*) can be collected by passing a cotton swab dipped in ethanol over the wing, which may also work with fleas (*Myodopsylla insignis).* Bat bugs (*Cimex adjunctus*) can be carefully 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 or scraped out, as well as fleas to be grabbed as they move quickly through the fur. Prior to SARS-CoV-2, blowing on the bat's fur assisted in moving it out of the way to see down to the skin without disturbing any potential fleas to be sampled. Alternative techniques described above (see 5.3.1 "Mist-nets") may serve a similar function.

9.0 Photography

Taking photographs may be warranted in the following circumstances: educational purposes, training (e.g., demonstrating handling and marking techniques on bats), species verification, obtaining wing morphology data, and documenting wounds, wing scarring from *P.d.,* traumatic injuries, or other unique features. It is important to remember that photography increases bat handling and holding times; 'trophy' pictures of bats are therefore discouraged. Tips for taking photographs of bats are available at www.whitenosesyndrome.org.

10.0 Euthanasia

Euthanasia is defined as a good death and, in the context of handling free flying bats, it means purposefully ending the life of an individual in such a way that minimizes or eliminates pain or distress (American Veterinary Medical Association (AVMA), 2020). Since the focus of this document is adherence to good principles of animal welfare, it is assumed that investigators and organizations participating in the activities described herein will have preplanned appropriately for the possibility of euthanasia during their work. This includes considering the appropriate endpoints for euthanasia, having the proper permits to allow them to perform euthanasia, receiving the appropriate training in the planned methods for euthanasia, and obtaining the necessary materials required for euthanasia prior to initiating their work. They will also have recognized that any procedure used for euthanasia must also prevent or minimize risk to the safety of personnel and the environment (AVMA 2020). Therefore, standard operating protocols (SOPs) for the best techniques of euthanasia should be developed by investigators and organizations as a method of ensuring a high standard of care for bats, adherence to good principles of animal welfare and appropriate biosafety measures are in place. The SOPS of investigators or organizations should be evaluated on a regular basis (i.e., every 2–3 years) to ensure they are current for training of personnel, document the most up to date techniques, and adopt in practice the most recent technologies and animal welfare practices.

Though rare, circumstances will arise during fieldwork when bats should be euthanized. For example, bats that are severely injured upon capture or during handling may not be suitable for release. Severe injuries could include, but are not limited to, fractures of the skull or large bones of the appendicular skeleton (e.g., humerus or femur), long tears in the skin of the body that cannot be immediately repaired, deep lacerations in the body wall with exposure of underlying organs in the body cavities, tears in the wing membranes that preclude normal flight, and significant bleeding (e.g., significant blood loss in a *M. lucifugus* is > 10% of body weight or ~1 ml). Bats with severe traumatic injuries may be found more commonly during surveys at wind turbines. Moribund or sick bats found on the ground and/or unable to fly can be captured and submitted to a wildlife veterinarian or a licensed wildlife rehabilitator for examination, but if one is not available, euthanasia is an appropriate consideration.

A technique chosen for euthanasia should cause rapid unconsciousness, followed by immediate cardiac or respiratory arrest and, ultimately, loss of brain function (Lollar 2018). Thus, euthanasia is frequently accomplished with a two-step process, first involving the use of an agent to depress or eliminate central nervous function followed by a second step to stop the heart (Sikes *et al.,* 2016). The animal becomes unconscious and insensitive to pain with the first action while the second method causes its death. Although it is possible to achieve both goals with a single agent, the primary concern is the increased time this might take and the need in some instances (e.g., severe traumatic injuries) to alleviate pain immediately (Sikes *et al.,* 2016). Therefore, the preferred and recommended method to achieve these results in the context of euthanasia for small insectivorous bats (i.e., ≤ 30 g) is to use an overdose of inhalant anesthetic gas followed by manual cervical dislocation to ensure death, whereas only an overdose of inhalant anesthetic gas is recommended for use in larger bats (CCAC, 2003). That said, data from domestic animals suggest manual cervical dislocation without the use of tools may be appropriate for rodents < 200 g (AVMA, 2020). Manual cervical dislocation may then be an appropriate secondary method to ensure the euthanasia of bats >30 g, namely *L. cinereus*, Canada's largest bat species weighing 35.7 g (Naughton *et.al.* 2012). **Therefore, it is strongly suggested to use the double method (i.e., an overdose of inhalant anesthetic gas followed by manual cervical dislocation) as the most appropriate technique for euthanasia of all Canadian bat species**. However, it is recognized that flexibility is required in fieldwork situations and some unforeseen events might result in manual cervical dislocation alone as being the only viable option for euthanasia.

An overdose of inhalant anesthetic gas in this instance involves the use of the volatile, pharmaceutical agent isoflurane in an airtight chamber (see 10.1 "Open-drop method", also known as “drop jar method”) to cause a generalized depression of the bat’s central nervous system, eventually leading to a cessation of breathing and death (Institutional Animal Care and Use Committee (IACUC), 2023; AVMA, 2020). Sevoflurane is a similar inhalant anesthetic gas, but it is not recommended for the open-drop method because its concentration cannot be accurately controlled with this procedure (IACUC, 2023). Isoflurane is not a controlled substance, but a prescription is required for its purchase. Therefore, it is necessary to acquire a prescription for the purpose of euthanasia, which can be obtained by developing a working relationship with a local, private veterinarian, a federal/provincial/territorial wildlife veterinarian, or your organizational/university veterinarian. Additionally, familiarization with the MSDS for isoflurane is necessary for biosafety to protect the personnel using it. Of utmost importance, isoflurane must be used only in a well-ventilated outside environment (or in a fume hood) because of the potential human health concerns associated with its use, especially for pregnant women. Therefore, a bottle containing isoflurane should never be transported in enclosed spaces, such as the cockpit or cabin of an aircraft or cab of a vehicle. It should instead be placed in an outside compartment of the transport vehicle (i.e., trunk, baggage compartment, or truck bed) within a closed, protective container, preferably encased in additional protective, shock absorbing material to prevent breakage of the bottle. Additionally, isoflurane should never be taken into enclosed spaces where bats are present such as hibernacula and roosts.

10.1 Open-drop method

The materials and equipment required for the open-drop method are minimal and include isoflurane, disposable gloves, protective eyeglasses, an airtight chamber of appropriate size for the target species, cotton balls, a 5ml syringe, and a firm, perforated metal container (e.g., tea ball strainer). For the body size of Canadian insectivorous bat species, a 250 ml to 500 ml chamber is of adequate size to hold an individual comfortably. It can be plastic or glass, but the bat to be euthanized should always be completely visible inside the chamber to assess signs of distress. Some plastics (e.g., hard, clear plastic) can melt on exposure to isoflurane and subsequently entrap the enclosed bat in the sticky, melted plastic causing it to become distressed (S. McBurney, pers. obs.). If a glass chamber is chosen, it must be carefully protected against breakage during transportation in the field. One of the authors (S. McBurney) has found 250 ml and 500 ml Mason (canning) jars well suited for this technique; when the rubber seal on the snap lid degrades (i.e., becomes sticky), it can be easily and affordably replaced by a new one.

The concentration of isoflurane required to euthanize a bat has not been reported, but a concentration of at least 5% anesthetic gas must be reached in a container to euthanize a bird (Daoust *et al., in preparation*). The IACUC (2023) reports that 0.25 ml of isoflurane in a 1 L container produces a 5% concentration of isoflurane (i.e., 0.0625mL isoflurane/250mL container or 0.125mL isoflurane/50 0mL container). However, if an investigator is in doubt about the volume of isoflurane to use, they should err on the side of a larger volume (Daoust *et al., in preparation*). Therefore, after donning disposable gloves and protective eyeglasses in a well-ventilated area, the investigator can use the syringe to draw 1–2 ml of isoflurane from its bottle and apply it in sufficient quantity to saturate a cotton ball that is held in the open tea ball strainer such that there is no resultant free-standing or dripping liquid. Subsequently, close the tea ball strainer prior to putting it in the chamber for holding the bat. This will prevent direct contact of the bat with the liquid isoflurane (IACUC, 2023), which is a reported skin and eye irritant (see product’s MSDS). When the bat is placed in the chamber, it should be monitored for any signs of distress. The chamber can be covered with a cloth or towel between observation periods to minimize stress. Allow a minimum of 15 minutes for the anesthetic overdose to result in unconsciousness and cessation of breathing. The bat can be left in the anesthetic chamber for a longer period to ensure the desired effect is achieved, but cessation of breathing is not a sufficient criterion of death and proper technique includes a follow-up examination to confirm death (Sikes *et al.,* 2016). Standard evidence of death in small insectivorous bats includes lack of withdrawal and palpebral reflexes (i.e., failure to respond to a toe pinch or touch of the eye respectively), as well as loss of muscle tone resulting in relaxation of wings and legs when extended. Even when all these criteria are met, the double method of euthanasia, which includes secondary manual cervical dislocation (see 10.2 "Manual cervical dislocation"), will ensure the bat's death.

The biology and ecology of bats raise additional considerations regarding the use of the open-drop method in these species. Bats that are in torpor either during hibernation or in cold ambient temperatures (usually $\leq$ 10ºC) have markedly reduced respiration so cessation of breathing can be difficult to assess. In such circumstances, it would be preferable to transport the bat to a well-ventilated, warm area (i.e., $\geq$ 20ºC) to bring it out of torpor prior to euthanasia. However, if this is not possible to accomplish in a timely, stress-free manner, manual cervical dislocation may be performed in the field as the sole method of euthanasia. Cold temperatures present in hibernacula and through the night at Canadian latitudes, even in the summer, may not allow isoflurane to sufficiently vaporize to be inhaled for the purposes of anesthetizing or euthanizing bats. In such circumstances, a similar approach to that described for bats in torpor can be employed as the most humane approach to euthanasia.

10.2 Manual cervical dislocation

As described above, manual cervical dislocation is preferably used as a second technique in the double method of euthanasia for bats, but it can be used as the primary technique by trained individuals in certain circumstances. This method separates the brain from the spinal cord and tears the blood vessels supplying the brain, resulting in 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 strength, and thus new practitioners should first practice on dead carcasses to ascertain that they can perform the technique, and that a certain degree of proficiency has been acquired to preclude distress (AVMA, 2020). Ideally the origin of the dead carcasses would be from bats that had recently died or had been euthanized with an overdose of inhalant anesthetic gas by a trained individual. This avoids the influence of rigor mortis and post-mortem decomposition which can markedly alter the anatomical feeling of the technique.

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 either side 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 above, observe the animal for lack of responsiveness and cessation of breathing, and confirm death with an appropriate follow-up examination.

10.3 Disposal of bats and waste materials

In the open-drop method after the bat is removed from the chamber and killed by manual cervical dislocation (see 10.1 "Open-drop method" and 10.2 "Manual cervical dislocation"), the chamber should remain closed until it can be placed in a secure, well-ventilated outside environment to allow complete evaporation of the remaining isoflurane. Once the cotton ball is dry, it can be disposed of in regular garbage and the chamber and tea strainer can be cleaned in hot water with an appropriate disinfectant. Other waste materials can be disposed of as biohazardous waste. Inhalant anesthetics can leave residues for days in euthanized animals, which is why their use for euthanasia is unsuitable for food-producing animals (AVMA, 2020). Therefore, bat carcasses euthanized with the open-drop method must be disposed of safely to prevent secondary toxicosis in animals of other scavenging species that may consume them or their parts. This is best accomplished by collecting and submitting them for veterinary health surveillance purposes (see 11.0 Health Surveillance). Even if manual cervical dislocation is the only method used for euthanasia, it is strongly recommended to collect and submit the dead bat for veterinary health surveillance purposes. If requested, this can include post mortem assessment of the head and neck to determine if an effective technique was utilized for the purposes of individual and/or organizational validation or improvement.

10.4 Unacceptable euthanasia techniques

Approaches to euthanasia that ignore recent advances in technology, and that do not minimize risks to animal welfare, personnel safety, and the environment for a particular set of circumstances, are unacceptable (AVMA, 2020). Therefore, several methods used for euthanasia of bats in the past are no longer considered acceptable practices. In general, these apply to most mammals and include air embolism, blow to the head, burning, chloral hydrate, cyanide, decompression, drowning, exsanguination (unless blood is collected from the unconscious animal as part of the approved protocol), formalin, various household products, hypothermia, neuromuscular blocking agents, rapid freezing, slow chilling freezing, strychnine, and stunning (Sikes *et al.,* 2011). Other methods specifically determined as unacceptable for bats for various reasons include 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 (Lollar 2018, Bat World Sanctuary (BWS), 2010).

11.0 Health Surveillance

Bats are cryptic species so sick or dead individuals are often hard to find. Projects that involve capturing and handling of bats provide a unique opportunity to identify appropriate specimens for health surveillance purposes. Several Canadian bat species are currently listed as Threatened or Endangered under SARA, thus it is important to identify health issues that might be contributing to, or causing, their decline. It is also imperative to determine anthropogenic causes of mortality so that prevention or mitigation strategies can be developed and implemented. Most importantly, research protocols and techniques can contribute to injuries and mortality in the study subjects. Therefore, it is incumbent on those involved to ensure animals they have captured and handled that subsequently die are necropsied to determine if anything related to the research procedure was the cause of their 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 in future work. Lastly, post-mortem examination of dead bats enables new and emerging health issues to be identified, particularly infectious disease problems (e.g., WNS most recently), and can lead to a better understanding of disease for the protection of bat and human health (e.g., rabies).

Investigators should report bats sighted outside hibernacula in the winter and sick, injured, or dead bats to local wildlife agencies to determine the best course of action. Often, agencies will suggest submitting dead specimens to your specific CWHC Regional Centre (http://www.cwhc-rcsf.ca/report_and_submit.php) or an alternative diagnostic laboratory for a complete post-mortem examination at no cost. To do this, fill out a submission form (downloaded from your CWHC Regional Centre's webpage) and follow the specimen shipping and handling instructions (http://www.cwhc-rcsf.ca/docs/CWHC%20Shipping%20and%20Handling%20Instructions.pdf). Ensure specimens are kept cool until shipped. If specimens must be held longer term, they can be frozen. Formaldehyde should not be used to preserve specimens. Many provinces and territories have a Wildlife Act that regulates the import and export of wildlife including bats and thus may require a permit to move these specimens. Please consult your local jurisdiction for policy and associated permits to legally import and export of wildlife specimens and samples.

If you have any questions about a sick or dead bat, you can call or email staff at a CWHC Regional Centre to get the appropriate guidance or additional information.

12. Knowledge Gaps

- Wing slit for bands: some practitioners have used this practice to improve banding of migratory bats. However long-term outcomes are unknown and warrant investigation.

- Use of antibiotics: The unanticipated, negative consequences of antibiotic resistance from indiscriminate use of antibiotics is well-documented and should be the most important consideration when contemplating the potential use of antibiotics. In the techniques described above, antibiotics are not considered necessary. However, some practitioners have placed small amounts of antibiotics on the open wounds of bats to prevent infection and promote healing. The effectiveness and potential risk to bat welfare are not known. It is likely affected individuals, as well as group members, would ingest anything applied topically. Therefore, it is unlikely that any topical application would be beneficial. Moreover, most topical treatments require repeated application to be effective. At the same time, any ill effects (if any) following ingestion are unknown. Non-sticky manuka honey may be a viable alternative, which comes as a cream. This has the benefit of killing bacteria, is not harmful if ingested, and does not promote antimicrobial resistance.

- Juvenile stress: it has often been assumed that juveniles are more susceptible to stress during capture and handling. However, there is no known research to support or refute this assumption and therefore further study is warranted.

- PIT tag wound healing and hair regrowth in fall/winter: it is generally assumed that healing and hair growth are limited in fall and winter when bat metabolism and immune systems are downregulated during torpor. However, there is no known research to support or refute this assumption and therefore further investigation is warranted.

- 5% rule: historically the 5% rule has been applied when attaching radio-transmitters to bats. However, it is not clear how well this rule can be applied across species and demographics with very different wing loading and morphology that will affect ability of bats to carry these loads.

- Band size: a band diameter approximately 7% the length of the forearm has been used by some investigators when selecting appropriate band size. However, there are currently no empirical data to support this practice. Further investigation is therefore needed.

- Predation risk associated with light tags: some evidence suggests light tags can remain attached and glowing for at least 48 hours. Whether or not this could attract predators remains unknown at this time.

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Appendix I. Bat Capture Survey Equipment Suppliers

Netting

- Avinet & Avian Research Supplies 276 Canco Rd. Portland, ME 04103 https://www.avinet.com/en/mist-nets/usa
- Bat Conservation & Management 1263 Claremont Dr. Carlisle, PA 17015 https://batmanagement.com/
- Ecotone Stryjska 24, 81-506 Gdynia, Poland http://www.mistnets.com/ultra_mistnets.html
- Ron Redman (Arkansas) – triple-high pole sets batman72015@yahoo.com
- Titley Scientific https://www.titleyscientific.com/us/ www.BatNets.com
- Faunatech Austbat 1055 Bullumwaal Road, Mountain Taylor, 3875, Victoria, Australia http://www.faunatech.com.au/products/harptrap.html?fbclid=IwAR3FX_KZfKgnw1nwi_E3nO1hrWljUIgpERbxN9TDPGpUYEFHrCbZ5GEL1aU

Holding bags

- Avinet & Avian Research Supplies 276 Canco Rd, Portland, ME 04103 https://avinet-avian-research-supplies.myshopify.com/collections/banding-supplies-tools/products/holding-bag-various-patterns-and-colors
- NHBS Ltd 1-6 The Stables, Ford Road, Totnes, Devon, TQ9 5LE, UK https://www.nhbs.com/1/bat-holding-bags?q=&fR[hide][0]=false&fR[live][0]=true&fR[shops.id][0]=1&fR[subsidiaries][0]=1&hFR[subjects_equipment.lvl1][0]=Bat%20Survey%20%26%20Monitoring%20%3E%20Bat%20Worker%27s%20Accessories%20%3E%20Bat%20Handling%20%26%20Measuring%20%3E%20Bat%20Holding%20Bags
- Legend Wholesale Distributors of Products to Mining Professionals 988 Packer Way, Sparks, NV 89431 Legend Heavy Duty Cloth Bags : Legend Inc. Sparks, Nevada USA (lmine.com)

Banding

- Porzana Ltd. Elms Farm, Pett Lane Icklesham, East Sussex TN36 4AH, UK http://www.porzana.co.uk/bat_rings.html
- BATS Research Center 107 Meadow View Court Shohola, PA 18458-3444 Office & Fax 570-409-0395 batresearch@yahoo.com contact: John Gumbs

PIT tagging

- Avid Identification Systems, Inc. 185 Hamner Ave. Norco, CA 92860 https://avidid.com/solutions/wildlife
- Biomark 705 S. 8th St. Boise, ID 83702 https://www.biomark.com/contact/
- Identification Solutions www.uidevices.com

- Oregon RFID 4246 SE Ogden St. Portland, OR 97206-8452 https://www.oregonrfid.com/
- Trovan Ltd. http://www.trovan.com/en Eidap Inc. Tel: +1 780 467 2707 Fax: +1 780 467 5160 Email: info@eidap.com Pacific Veterinary Sales Tel: +1-800-663-6644 Fax: +-877-850-1510 Email: trevor@pacificpet.net
- Manruta Room 2011, Zhong Zin Ming Zuo Building, Zhongxin Rd, Shaijing Town, Bao'an Distinct, Shenzhen, Guandong, China 518104 http://www.manruta.com/?fbclid=IwAR3MLcL5fbk2LBAHD0MDviJYbrx0QM-iCILObTA3Ehily1NO3oOut3WrN6A

Biopsy punches

- Stevens Medical Supplies 8188 Swenson Way Delta, BC V4G 1J6 https://stevens.ca/
- Surgo Surgical Supply http://www.surgo.com/z_homepage.htm

Radio-telemetry

- ATS Track 470 First Ave. NW Isanti, MN 55040 https://atstrack.com/trackingsystems/entomologists-and-bat-package.aspx
- Lotek Wireless Inc. 115 Pony Dr. Newmarket, Ont. L3Y 7B5 http://www.lotek.com/avian.htm
- Holohil Systems Ltd. 112 John Cavanaugh Dr. Carp, Ont. K0A1L0 https://www.holohil.com/

Bat detectors

- Land-based Learning https://landbasedlearning.ca/shop/
- Wildlife Acoustics https://www.wildlifeacoustics.com/
- Titley Scientific https://www.titley-scientific.com/us/
- Pettersson Electronik https://batsound.com/
- Bat Conservation and Management https://batmanagement.com/