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Hummingbird (Family Trochilidae) Research: Welfare-conscious Study Techniques for Live Hummingbirds and Processing of Hummingbird Specimens

Lisa A. Tell, Jenny A. Hazlehurst, Ruta R. Bandivadekar, Jennifer C. Brown, Austin R. Spence, Donald R. Powers, Dalen W. Agnew, Leslie W. Woods, and Andrew Engilis, Jr.

Dedications

To Sandra Ogletree, who was an exceptional friend and colleague. Her love for family, friends, and birds inspired us all. May her smile and laughter leave a lasting impression of time spent with her and an indelible footprint in our hearts.

To my parents, sister, husband, and children. Thank you for all of your love and unconditional support.

To my friends and mentors, Drs. Mitchell Bush, Scott Citino, John Pascoe and Bill Lasley. Thank you for your endless encouragement and for always believing in me.

~ Lisa A. Tell

Front cover: Photographic images illustrating various aspects of hummingbird research. Images provided courtesy of Don M. Preisler with the exception of the top right image (courtesy of Dr. Lynda Goff).

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Museum of Texas Tech University
Lubbock, TX 79409-3191 USA
(806)742-2442

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Abstract

Research on hummingbirds over the decades has provided insights into their evolution, migration, physiology, and numerous other areas, including conservation biology. Their small size, energy demands, and high metabolic rates are some of the challenges researchers face when obtaining research samples and biologic materials from live hummingbirds. This manuscript summarizes the established literature dealing with basic methods that scientists have used when capturing, handling, and otherwise researching hummingbirds. Based on the authors' experience, best practices for working with live hummingbirds are presented, including permitting requirements for studying live hummingbirds, trapping and marking, handling techniques, safe collection of tissue samples, first-aid measures, and euthanasia of hummingbirds, as well as processing of hummingbird specimens (e.g., necropsy and preservation).

Key words: bleeding, capturing, euthanasia, handling, marking, metabolic rates, museum specimens, permitting, restraining, sampling

Introduction

Numerous species of hummingbirds (Family Trochilidae) have been the subjects of scientific investigations involving evolution, behavior, physiology, flight mechanics, feather structure, diseases, and conservation biology. In addition, due to an increasing interest in pollinators, hummingbird research appears to be on the rise. However, given their small body size and high metabolism, obtaining samples from live hummingbirds can pose challenges, especially for novice investigators. Sampling techniques have been well established for other avian species, but these methods are not always transferable to hummingbirds. To continue advancing scientific discovery for this unique family of birds, this manuscript provides guidelines for obtaining study permits, as well as safe, efficient, and ethical methods of sampling live hummingbirds while maintaining high standards of animal care and welfare. Recommendations for dealing with injured or extremely ill birds are addressed, along with protocols for euthanasia. Necropsy techniques, specimen preparation for museum vouchering, and organ and muscle sampling also are described in detail.

The study of wild birds remains essential for ornithologists who are trying to understand, under natural conditions, basic questions of ecology, physiology, disease, and behavior. Capture, restraint, and sampling of hummingbirds have led to a better understanding of their physiology and flight mechanics (Chai et al. 1996; Warrick et al. 2005; Bakken and Sabat 2006; Welch et al. 2007), evolution of biodiversity and mutualisms (Chaves and Smith 2011; González-Gómez et al. 2014a., b; Maglianesi et al. 2014a, b; Ornelas et al. 2014; Abrahamczyk and Renner 2015; Gonzalez and Loiselle 2016), disease prevalence (Godoy et al. 2013, 2014; Backus et al. 2019; Magagna et al. 2019; Baek et al. 2020), and taxonomy and systematics (McGuire et al. 2009, 2014). Much of this research has been achieved through obtaining biological samples such as blood, muscle, organ tissues, excreta, or feathers from live hummingbirds or preserved specimens.

Although there is much to be gained scientifically by studying hummingbirds, striving for a balance between sampling risk and scientific gain is paramount.

Risks can be minimized if researchers are vigilant about the working environment, the hummingbirds' condition, the amount of handling time, and employment of welfare-oriented best practices. Most researchers focus on the obvious small size and delicate skeletal frame when working with hummingbirds. North American hummingbirds range in weight from approximately 2.3 g (Calliope Hummingbird, *Selasphorus calliope*) to 8.6 g (Blue-throated Mountain-gem, *Lampornis clemenciae*). However, when working with hummingbirds, many other factors must be considered. One factor is the high mass-specific metabolic rate of these species compared to those of other vertebrates (Suarez 1992). Given their energy demands, hummingbirds operate at the limits of their metabolic needs. Therefore, when handling and restraining hummingbirds, investigators must be mindful of their caloric needs to ensure successful release. Additionally, hummingbirds have the lowest mass of feathers by weight of any bird. Their lack of down feathers (King and McLelland 1984) prohibits over-sampling of feathers, which may alter a hummingbird's ability to thermoregulate and increase the risk of hypothermia (particularly for species inhabiting high elevations). For some hummingbird species, tail feathers also play an acoustic role during courtship displays (Clark et al. 2018a; Clark and Mistick 2018a); therefore, sampling certain feathers during the breeding season may alter reproductive fitness.

As studies incorporating live hummingbird sampling become more common, investigators should examine and consistently re-evaluate techniques to maintain good practices for conducting high-quality and ethical research. This manuscript summarizes the established literature that details basic methods used by banders and scientists who work with hummingbirds and then suggests best practices for working with live hummingbirds. In addition, guidelines are provided for optimizing the utility of birds that have died or are collected for specimen archiving.

There are numerous approaches for achieving similar outcomes; therefore, the proposed techniques should be viewed as based on experience and not as definitive or exhaustive in nature. Resources and recommendations listed in this manuscript are intended to provide new investigators with baseline information and offer experienced researchers alternative options.

1. Laying the Groundwork: Study Approval and Permitting

1.1. Literature Search

Performing a thorough literature search prior to sample or specimen collection minimizes scientific duplication and maximizes efficiency so as to reduce the potential impact on study subjects. A literature review will be required by an Institutional Animal Care and Use Committee (IACUC) if the investigator is employed by an institution that receives federal funding for laboratory research and performs research on certain animal species (see section 1.2). Scientists also should supply background scientific information when applying for federal or state permits. Literature searches for hummingbird-related publications can be performed using conventional biological literature databases. The Cornell Lab of Ornithology (http://www.birds.cornell.edu/Page.aspx?pid=1478) has avian-related resources available, and Partners in Flight (http://pif.birdconservancy.org/#) has population estimates and avian conservation assessment databases. Ornithology Exchange has a listing of ornithological journals (https://ornithologyexchange.org/resources/journals/database/ornithological-journals/) and other journals of interest to ornithologists (https://ornithologyexchange.org/resources/journals/database/other-journals/).

For this manuscript, a reference library of publications was compiled using search terms "hummingbird" OR "hummingbirds" OR "Trochilidae." Searched databases included SciELO, Web of Science, BIOSIS Previews, CAB Abstracts, Scopus, EBSCOhost, and PubMed. Google Scholar was not included because the search algorithm is not transparent, and results can contain more false drops (items found that are not related to the search topic) than useful records. Once the reference library was established, studies were evaluated and selectively summarized in tables so that readers could see the variety of sampling methods that have been published in peer-reviewed articles. Manuscript-reported numbers for these tables represent unique studies where individual publications were counted only once but appeared multiple times in

one table. This library is available on-line at (https://www.zotero.org/ucdhummingbirdhealthbibliography/library). As of September 2020, the library contained 2,039 citations.

The following subsections of this manuscript discuss pre-study requirements for virtually all types of research on hummingbirds. Such prerequisites include following IACUC standards and obtaining permits allowing the capture and processing of hummingbirds. Depending on the focus of the study, the researcher may be required to obtain permits issued by the United States Geological Survey (USGS) Bird Banding Laboratory (BBL), the United States Fish and Wildlife Service (USFWS), the state in which the study is performed, and, potentially, additional authorities.

1.2. Institutional Animal Care and Use Committee and Protocols

The Institutional Animal Care and Use Committee is appointed to oversee research activities affiliated with an institution in the United States (US) of America. This committee ensures that all procedures comply with the Animal Welfare Act (AWA 1966, 1970; AWAR 2020) and Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS 2015). Investigators working at research institutions that receive US federal funding must apply for IACUC protocol approval for any proposed research project that involves animals or animal sampling. Protocols are evaluated for scientific merit, animal welfare, procedure appropriateness, and animal care and maintenance. For IACUCs that evaluate research protocols involving hummingbirds, this manuscript could be used as a reference for best practices. Other helpful references that summarize concepts and provide guidelines for using wild birds in research include Wild Bird Guidelines for Research (Fair and Jones 2010) and the Report of the Committee on Use of Wild Birds in Research (Oring et al. 1988). In addition, Espin et al. (2014, 2020) summarized fundamental sampling and sample storage protocols for raptors, some of which could be applied to hummingbird research.

1.3. Permit Requirements

Because the authors work and have the most expertise in the United States of America, the permit section of this manuscript is limited to this geographic region. Permit requirements to work with live birds differ for Canada, Mexico, and Central and South America and have not been addressed herein. Import/export permit requirements also vary worldwide. Permitting requirements detailed herein reflect policies that were in place at the time of publication but are subject to change. Researchers should routinely consult the websites of federal and state agencies for current permit requirements. Website addresses (URLs) for federal agencies are referenced in the footnote section of Table 1.

The protected status of the hummingbird species that will be captured, restrained, banded, marked, and/or sampled is a key component to the permitting process. All hummingbird species are listed in Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES). CITES is an international agreement that serves to protect wild animals and plants from international trade that might threaten their survival. The checklist for CITES species can be found at http://checklist.cites.org/#/en. Some hummingbird species also are listed under the Endangered Species Act (ESA). ESA species listings can be found at the website of the USFWS Environmental Conservation Online System (ECOS) at https://ecos.fws.gov/ecp/. At the time this manuscript was prepared, none of the hummingbird species that are found in or migrate through North America were ESA listed. Hummingbirds found in North America have protected status under the Migratory Bird Treaty Act (MBTA 2020). In addition, special status species may require additional justification and permitting restrictions under permitting programs. Federally designated "Birds of Conservation Concern" (USFWS Birds of Conservation Concern: https://www.fws.gov/birds/management/managed-species/birds-of-conservation-concern.php) or state-designated "Species of Special Concern" (i.e., California Species of Special Concern: https://wildlife.ca.gov/Conservation/SSC or Nevada Species of Conservation Priority: http://www.ndow.org/Nevada_Wildlife/Conservation/Nevada_Wildlife_Action_Plan/) are examples of special status species.

In the United States of America and its territories, activities associated with hummingbird research require federal and often state permits (Table 1). Federal permits are required when working with live or

Table 1. Required federal (United States Fish and Wildlife Service [USFWS] or United States Geological Survey [USGS] Bird Banding Laboratory [BBL]) or state permits for conducting hummingbird research in California, United States of America.[+]

Process	Method	USFWS	BBL	State
Capturing and marking a hummingbird	mist-net, drop-door, etc.	N	Y	Y
Capturing and *not* marking a hummingbird	mist-net, drop-door, etc.	Y	N	Y
Holding a hummingbird in captivity for ≤ 24 hours (only for the safety and well-being of an individual bird; not for sample collection or procedures relative to research)	any method	N	Y	maybe
Holding a hummingbird in captivity for ≤ 24 hours (for research purposes, including but not limited to sample collection)	any method	Y	N	Y
Holding a hummingbird in captivity for > 24 hours	any method	Y	N	Y
Auxiliary marking of a hummingbird[++]	color marking, tagging	N	Y	Y
Banding of a hummingbird	banding	N	Y	Y
Feather sampling and banding/marking	any feather	N	Y	Y
Feather sampling and *no* banding/marking	any feather	Y	N	Y
Blood sampling and banding/marking	any method	N	Y	Y
Blood sampling and *no* banding/marking	any method	Y	N	Y
Cloacal or oral swab sampling and banding/marking	swab	N	Y	Y
Cloacal or oral swab sampling and *not* banding/marking	swab	Y	N	Y
Fecal/urine collection that requires restraint or containment of the bird	restraint or contained holding of a bird	Y	N	Y
Fecal/urine collection *after* the sample has left the bird's body and without restraining or retaining the bird	fecal/urine sample collection from an abandoned nest or under a free ranging roosting area etc.	N	N	maybe
Euthanasia due to injuries or pre-existing conditions	IACUC approved	maybe*	Y**	Y
Lethal take of birds (healthy birds for study skins, invasive sample collection, disease studies, etc)	IACUC approved	Y	N	Y

Table 1. (cont.)

Process	Method	USFWS	BBL	State
Treatment of injured bird (during processing or pre-existing condition)	IACUC approved	N	N	N
Salvage provisions for bird mortality occurring in association with banding/marking activities	possession for a period up to 6 months post death	N	Y	maybe
Salvage activities unrelated to bird banding/marking	possession length dependent on details listed in permit	Y	N	maybe

+Hummingbirds are protected under the US Migratory Bird Treaty Act (MBTA 2020). At the time that this manuscript was written, no hummingbird species found or traveling through North America appeared on the federal list of endangered and threatened species. Should a hummingbird species, subspecies, or population be added to that list, then a researcher proposing to study that species must obtain a USFWS Endangered Species Section 10 Recovery permit in addition to the permits listed in this table. The USFWS Endangered Species program should be consulted for these permitting requirements. The Endangered Species Recovery permit must be obtained before the BBL will approve authorizations on a banding permit to capture, band, and/or mark any federally-listed species.

++Auxiliary marking refers to any color or identifying tag or device other than a federally-issued bird band and includes any color marking or tag, for example a very high frequency or radio-frequency identification tag.

* If an individual has a USFWS Scientific Collecting permit, then they may only euthanize a bird if lethal take of that species is authorized by the permit.

**This is limited to an individual holding a BBL master permit or sub-permit being able to conduct euthanasia only when a hummingbird is injured during permit approved activities and the permit holder determines that euthanasia is the only proper action given the extent of the bird's injuries. The BBL permit does not provide general authorization to euthanize birds and should not be used to euthanize a perfectly healthy bird. The BBL general permit conditions are: If a bird is found injured or severely diseased, the bander must assess the situation and determine if treatment and rehabilitation would lead to the bird's recovery. If it is likely that treatment will allow the bird to recover, the bander should transport the injured bird to an avian rehabilitation facility. If the BBL permittee determines that recovery is not likely given the extent of injuries or disease, they should euthanize the bird using techniques as advised by the American Veterinary Medical Association or American Ornithological Council. BBL permittees operating under an institutional animal care and use committee (IACUC) approved protocol need to use IACUC authorized euthanasia methods.

Note: For current regulations and guidelines, it is advised to routinely consult the United States Geological Survey Bird Banding Laboratory (https://www.usgs.gov/centers/pwrc/science/bird-banding-laboratory?qt-science_center_objects=0#qt-science_center_objects) and the United States Fish and Wildlife Service (https://www.fws.gov/birds/policies-and-regulations/permits/permit-policies-and-regulations.php) websites.

dead hummingbirds, and nearly every state also has a separate and unique permitting process for working with migratory birds, including hummingbirds. The Ornithological Council provides guidelines, considerations, and links for state permitting at https://birdnet.org/info-for-ornithologists/permits/states/. Obtaining new or renewed permits can take considerable time (sometimes months to years) depending on the agency; therefore, it is important to plan accordingly. Due to variation in federal, state, tribal, and local laws, regulations, and policies, permit requirements from these entities also can vary. If there is a discrepancy between two separate permits, permittees should follow the most restrictive permit requirements.

Hard copies or readily accessible digital files of federal and state permits must accompany the investigator, master bander, or sub-permittees working in the field. Permit copies also must accompany samples during shipment or transport, including specimens donated to museums or research institutions. Best practice would be that prior to shipment, permits (if applicable) are exchanged between individuals sending and receiving the samples to ensure that proper documentation is in place. Depending on the specific situation, the researcher may require a permit from the BBL, USFWS, the state, and/or another authority.

1.3.1. United States Geological Survey Bird Banding Laboratory (BBL) permits.— Anyone in the US who is banding, marking, and handling hummingbirds independently in the field is required to possess either a valid BBL master permit or sub-permit (50 CFR § 21.22). United States BBL permit authorizations are normally restricted to activities occurring within the US and its territories. Under very limited circumstances, the BBL may issue authorizations to use BBL-issued bands outside of the US on species covered under the MBTA (MBTA 2020). Permits from the foreign country approving the use of BBL-issued bands in that country must first have been obtained and submitted to the BBL with a special circumstances request. An example would be a researcher wanting to conduct a study of Ruby-throated Hummingbirds (*Archilochus colubris*) and band them while in their wintering range in Central America. The researcher must first receive permit approval from the country in Central America to use the BBL-issued hummingbird bands, and then the BBL would consider granting approval. Further details can be found on the BBL website (https://www.usgs.gov/centers/pwrc/science/banding-foreign-countries?qt-science_center_objects=0#qt-science_center_objects).

The BBL issues master permits or sub-permits depending on the study proposal(s), an investigator's level of experience, and requested activities. Permits issued will specify: states in which the bander can work; hummingbird species with which the bander can work; blood, feather, and/or swab samples that are allowed to be collected; and capture techniques allowed for obtaining hummingbirds. All of these specifications will depend on the experience and training of the individual. When a field crew is conducting hummingbird-related activities, at least one crew member is required to have a BBL master permit or sub-permit. Other team members can legally capture, handle, band, or mark hummingbirds without an individual permit but only under the direct supervision of a BBL-permitted bander. If field team members are operating independently for any activities (e.g., capturing, handling, or banding), each member will need to be issued a BBL sub-permit. To obtain a master banding permit, a completed application must be submitted to the BBL permit office (https://www.usgs.gov/centers/pwrc/science/permit-application-instructions?qt-science_center_objects=0#qt-science_center_objects). Requests for sub-permittees need to be made by the master bander and submitted to the BBL permit office (https://www.usgs.gov/centers/pwrc/science/requests-sub-permits?qt-science_center_objects=0#qt-science_center_objects).

To be granted a BBL permit, an individual must provide evidence of their knowledge and skills used to capture, handle, band, and/or mark hummingbirds. Individuals initially are trained under the guidance and supervision of a BBL-permitted hummingbird bander. Trainees must also complete and pass a training course in hummingbird handling and banding techniques, including the process used to properly create hummingbird bands. Additional information can be found at https://www.pwrc.usgs.gov/BBL/homepage/species_auth.cfm. Such courses provide an independent assessment of a bander's knowledge and skills and must be completed before a request is submitted to the BBL to add hummingbird banding authorizations to a pre-existing BBL permit for other avian species.

Increasingly, graduate students are conducting research projects that require BBL permits; however, in some cases, faculty advisers have very limited or no banding experience. The BBL will normally recommend that these students first train under the supervision of an experienced and BBL-permitted passerine bander before starting their independent work with hummingbirds (B. Peterjohn, pers. comm., 28 June 2020). In addition, they are required to complete the aforementioned training on hummingbird-specific banding before they can begin their field activities. Because most of these training activities occur only during the summer, graduate students should plan accordingly. Once graduate students can demonstrate competence and obtain references from BBL-permitted banders, the BBL will consider issuing master BBL permits or sub-permits with "limited" authorization(s) tailored to the specific activities of the research project(s). Individuals wishing to attend a hummingbird banding training course should contact the BBL for more information, recognizing that these training activities are very limited.

Depending on the scenario, trapping, mist netting, and/or marking hummingbirds in the US requires either a BBL permit or a USFWS Migratory Bird Scientific Collecting permit. In addition, nearly every state has an agency with its own permitting requirements. Prospective researchers must submit an application to the appropriate federal and state wildlife agencies. In most cases, the federal permit(s) need(s) to be obtained before a state agency will consider a permit request. A BBL permit is needed for banding or marking hummingbirds, and authorized capture techniques would be included. For capturing and handling hummingbirds where banding or marking will not occur, a USFWS permit is required. Requirements for obtaining USFWS permits are discussed in the following subsection. A BBL permit also is required for anyone placing a federal bird band or auxiliary marker (e.g., a color band, radio-frequency identification tags, or geolocators) on a bird that will be released into their natural habitat. Investigators marking a free-ranging bird (e.g., feather dying, applying paint, or notching feathers) without placing a federal bird band or an auxiliary marker should contact the BBL for guidance on permit requirements.

Birds that are hatched in captivity or caught in the wild with the intent of being permanently housed in captivity should not be banded with a BBL-issued band (B. Peterjohn, pers. comm., 28 June 2020). In the case of a banded free-ranging bird that is converted to being permanently held in captivity, the BBL band should be removed if removal can be performed safely. Otherwise, the BBL band can remain on the bird. If a hummingbird is hatched, raised, and retained in captivity and needs to be individually identified for sampling purposes, BBL federally issued bands cannot be used. One option is to contact the BBL and arrange for bands to be made specifically for the captive bird population using a different letter prefix than those used for free-ranging birds (B. Peterjohn, pers. comm., 28 June 2020).

BBL permit authorization to band and sample rehabilitated birds also is allowed. Similar to master or sub-permittee requirements, experience identifying, aging, and sexing the requested hummingbird species must be documented. However, documenting capture technique experience is not required because birds will already be in captivity at the time of banding. Because hummingbirds require specialized bands, the applicant also must have experience with hummingbird banding protocols. The BBL requires submission of a project proposal that details the scientific or conservation merit for banding and sampling rehabilitated birds (https://www.usgs.gov/centers/pwrc/science/banding-rehabilitated-birds?qt-science_center_objects=0#qt-science_center_objects).

Individuals banding or marking hummingbirds also can apply to collect blood, feather, and/or swab samples, and such authorizations will be included on their BBL permit. Anyone wishing to collect blood samples from hummingbirds must receive training in blood sampling techniques before the BBL will approve that authorization on a banding permit. Because specialized techniques are required to obtain blood samples from hummingbirds, experience obtaining blood samples from other birds is not necessarily applicable to hummingbirds. Training in hummingbird blood sampling techniques is currently very limited, and the BBL should be contacted for additional information (https://www.usgs.gov/centers/pwrc/science/blood-sampling?qt-science_center_objects=0#qt-science_center_objects and Bruce Peterjohn, pers. comm., 28 June 2020). Although swab sampling authorizations

are issued by the BBL, such requests require specific details, such as the size of the swab to be used and the anatomic site to be swabbed. Because there are no published methods for safely taking swab samples from the oral cavity or cloaca of live hummingbirds, swab authorization requests are likely to be viewed as experimental procedures. Such requests must be accompanied with detailed descriptions of the proposed sampling protocol(s) before the BBL will consider approval (B. Peterjohn, pers. comm., 28 June 2020).

For a bird to be banded and/or marked and restrained and/or held in confinement for collection of any other type of biological sample (other than blood, feather, and/or swab samples), both BBL and USFWS Migratory Bird Scientific Collecting permits are required (see https://www.fws.gov/migratorybirds/pdf/policies-and-regulations/3-200-7FAQ.pdf). If samples authorized on a USFWS Migratory Bird Scientific Collecting permit (e.g., urine, fecal, or lesion scraping samples) are collected, and the bird is banded and trapped/released during the event that had the sole purpose of urine, fecal, or lesion scraping sampling, then the bird should be reported as banded under the BBL permit and reported as trapped and released on the USFWS scientific collecting annual report form (3-202-1; https://www.fws.gov/forms/3-202-1.pdf). BBL permittees must adhere to the BBL requirement that healthy birds be released immediately following completion of banding, marking, and blood, feather, and/or swab collection. Birds cannot be restrained, held, or confined for any activities outside of banding, marking, or obtaining blood, feather, and/or swab samples under the BBL permit unless they have a USFWS Migratory Bird Scientific Collecting permit that allows further containment (50 CFR § 21.23; B. Peterjohn, pers. comm., 28 June 2020). Cloacal excrement samples can be collected opportunistically (e.g., from a bird holding bag) while a hummingbird is being processed (i.e., banded, bled, or feather sampled) under a BBL permit. However, hummingbird processing must be performed in a timely fashion and birds retained for reasonable lengths of time (i.e., no longer than 15 min). Containment should not be extended to advantage opportunistic sampling of cloacal excrement.

In the case of sick or injured hummingbirds, a BBL permit authorizes permittees to hold such birds in captivity for up to 24 h, but only for the purpose of ensuring the safety and well-being of the bird and long enough for the bird to recover or be transported to a wildlife rehabilitator (B. Peterjohn, pers. comm., 28 June 2020). This provision does not allow an investigator to opportunistically collect samples (e.g., urine, feces) during containment, because the focus should be on supporting the bird and minimizing disturbance prior to timely release or transfer to a wildlife rehabilitator.

If bird mortality occurs during banding, marking, and/or blood or feather sampling, the BBL permit serves as a salvage permit allowing dead birds to be kept up to six months. Salvage activities unrelated to bird banding, marking, or authorized BBL sample collection procedures must be covered under a USFWS Migratory Bird Scientific Collecting permit (that includes salvage authority) and likely under state permits as well.

All permitting requirements described herein reflect BBL policies at the time of publication; however, these requirements could change. Thus, researchers should always consult the BBL website for the most up-to-date banding permit requirements. Further details regarding BBL permit requirements can be found on the general permit information section of the BBL website (https://www.usgs.gov/centers/pwrc/science/general-permit-information?qt-science_center_objects=0#qt-science_center_objects).

1.3.2. United States Fish and Wildlife Service (USFWS) permit.—A USFWS Migratory Bird Scientific Collecting permit authorizes qualified individuals to collect (live, lethal, and/or salvage), transport, or possess migratory birds, their parts, nests, or eggs for scientific research or educational purposes (50 CFR § 21.23). USFWS Migratory Bird Scientific Collecting permits also may authorize importing or exporting of birds and/or bird samples into or out of the United States; however, an investigator must request to add this condition to their permit (https://www.fws.gov/migratorybirds/pdf/policies-and-regulations/3-200-7FAQ.pdf).

A scientific collecting permit from a USFWS Migratory Bird Permit Office (https://www.fws.gov/forms/3-200-7.pdf) is required if live birds will be: (1) captured, restrained and/or contained and released;

(2) sacrificed (lethal take); or (3) captured from a free-ranging environment then held in captivity. If a hummingbird is captured for blood, feather, or cloacal/oral swab sample collection and not banded and/or marked, then a BBL permit is not required; rather, a USFWS Migratory Bird Scientific Collecting permit is required and likely a state permit as well. In addition, a USFWS Migratory Bird Scientific Collecting permit is necessary for the capture, restraint, and/or retention of a hummingbird for the purposes of urine and/or fecal sample collection. Even though collection of urine and fecal material do not fall under the purview of either the BBL or USFWS Migratory Bird permits, the capture, restraint, and/or retention activities do. If urine and fecal samples are collected after the urine or feces exit the cloaca and the hummingbird producing the excrement is not captured, restrained, or contained to obtain the samples, BBL or USFWS Migratory Bird permits are not required; however, a state wildlife agency permit might be necessary.

When applying for a USFWS Migratory Bird Scientific Collecting permit, the investigator needs to declare where live birds, samples, or specimens obtained by lethal collection will be housed or stored. Carcasses or any remaining materials from a specimen must be donated to a public scientific or educational institution upon conclusion of the research project. It is recommended that the investigator work with a regional or state museum to establish vouchering protocols that can be included in permit applications.

USFWS Migratory Bird Scientific Collecting permits include salvage authorization. Salvaged specimens can be a bird and/or bird components (i.e., feathers, wing, skeleton) opportunistically found in the wild, birds that have died at rehabilitation centers, and/or bird specimens and/or bird components that are being donated by a researcher after completion of a scientific project. Salvaging is defined as collecting a deceased bird when the collecting individual has no involvement in the death of the bird. Salvaged specimens can be accepted by a curator or researcher at an accredited museum or other authorized institution. Salvage for other purposes may be authorized under a USFWS Special Purpose – Salvage permit (50 CFR 21.27; Form 3-200-10a; https://www.fws.gov/forms/3-200-10a.pdf). A USFWS Migratory Bird Scientific Collecting, Special Purpose – Salvage, or Special Purpose Possession for Education permit is needed to salvage nests, eggs (viable or non-viable), or dead birds that the researcher had no part in euthanizing or lethally collecting. The type of permit depends on the type and purpose of the activity. Additional information about Special Purpose Possession for Education permits (Live and/or Dead Possession: Form 3-200-10c) and Special Purpose Salvage permits (Form 3-200-10a) can be found in the Frequently Asked Questions associated with the permit applications on the USFWS Migratory Bird Program website (https://www.fws.gov/birds/policies-and-regulations/permits/need-a-permit.php).

Public scientific or educational institutions can accept migratory bird specimens for research and educational use that were lawfully collected, and those institutions do not need a permit to possess these specimens (50 CFR § 21.12(b)(1)). The definition of "public" can be found in 50 CFR § 10.12 and is as follows: "Public as used in referring to museums, zoological parks, and scientific or educational institutions, refers to such as are open to the general public and are either established, maintained, and operated as a governmental service or are privately endowed and organized but not operated for profit." Private scientific and educational institutions also can accept and possess migratory bird specimens that were lawfully acquired, without themselves needing a USFWS Migratory Bird Scientific Collecting permit; however, a Special Purpose Possession for Education permit would be necessary.

To obtain a USFWS Migratory Bird Scientific Collecting permit, applications must be submitted to the USFWS Migratory Bird Permit Office that oversees activity in the geographic area where the researcher lives (https://www.fws.gov/birds/policies-and-regulations/permits/regional-permit-contacts.php), not where the research is to be performed. For USFWS permits, if the permit renewal application is postmarked 30 days before the current permit expires, permittees may continue activities authorized by their permits until the USFWS has acted on the renewal request (50 CFR § 13.22). New activities will require approval. If the deadline is missed and the permit expires, the permittee will be required to submit a new application rather than submitting the signed renewal letter that the Migratory

Bird Permit Offices currently provides to permittees. The USFWS is currently in the process of developing an online permitting system that is expected to be available by the end of September 2020. This new online system will allow permit applications to be submitted electronically. Until the online permitting system is available, the Migratory Bird Scientific Collecting permit application (Form 3-200-7) is available on the USFWS website (https://www.fws.gov/forms/3-200-7.pdf) and may be printed and mailed to the USFWS, Migratory Bird Permit Office. Table 2 provides expanded explanations of required information needed for a Migratory Bird Scientific Collecting Permit Application (Form 3-200-7; http://www.fws.gov/forms/3-200-7.pdf) or for a renewal application for a Migratory Bird Scientific Collecting Permit. These items are not an exhaustive list of application components but provide guidance for expectations.

1.3.3. State permits.—Scientific collecting permits or research permits are required by state agencies regulating wildlife-related activities. The Ornithological Council website has a section that summarizes state permitting requirements and provides the names for the appropriate contact in each state (https://birdnet.org/info-for-ornithologists/permits/states/). State permits must accompany the appropriate federal permit(s). Researchers should understand local conservation and protection laws governing hummingbirds at their study sites.

1.3.4. Additional permit authorizations.—Any scientific, banding, and/or marking activities conducted on federal, state, or public lands may require a permit from the agency administering those lands. Examples of federal lands that might include special use permits are USFWS National Wildlife Refuges, US Forest Service, or Bureau of Land Management specially designated areas. Public properties administered by county or regional authorities and land maintained by non-governmental organizations (e.g., Nature Conservancy reserves) also may have special permitting requirements. Each agency/land management organization should be contacted regarding their specific permitting requirements. The Ornithological Council provides information and links to federal agencies that require permits when a researcher is working on federally owned lands (https://birdnet.org/info-for-ornithologists/permits-us-federal/). The authors are unaware of a similar resource for permitting requirements by state agencies. Any work conducted on private lands must be with landowner permission, and written consent is highly recommended.

2. WORKING WITH LIVE HUMMINGBIRDS

2.1. Hummingbird Identification in the United States of America

Proper identification of the species, age, and sex of a hummingbird is critical for scientific studies. The following guides have been found to be most useful for providing identification data: *Identification Guide to North American Birds Part 1* (Pyle 1997); *A Field Guide to Hummingbirds of North America* (Williamson 2001); *Hummingbirds of North America: The Photographic Guide* (Howell 2002); and the *North American Banders Manual for Banding Hummingbirds* (Russell and Russell 2019). Because identification of hummingbird species, age, and sex can be challenging, it is helpful for researchers to carefully study reference guides and practice with museum study skins before working with live birds. This will help minimize restraint time and live bird energy expenditure and is especially important for novice investigators or those unfamiliar with species in a newly studied geographic area. Voucher specimens are most likely to be found at natural history museums, where access policies vary.

Researchers undergoing training to become hummingbird banders should learn traits of the research species and/or traits of the suite of species in the geographic area where they will be working. Researchers also should become familiar with traits of species within the same genus, even if these species are not commonly found in the geographic area of interest, so that unusual encounters can be properly identified. BBL permittees working with unfamiliar hummingbird species also should be encouraged to receive training from permittees that are experienced with the species

Table 2. Required or optional study proposal components for a Migratory Bird Scientific Collecting permit. The items below are expanded explanations of information required for a Migratory Bird Scientific Collecting permit (Migratory Bird Scientific Collecting Permit Application; Form 3-200-7 found online at http://www.fws.gov/forms/3-200-7.pdf) and Migratory Bird Scientific Collecting permit renewal letters (mailed or emailed by the Migratory Bird Permit Office). These components also need to be provided with permit amendment requests. This is not an exhaustive list of required application components, and investigators are encouraged to fully review Form 3-200-7 to evaluate which components might be necessary for their studies.

Component	Required or Optional	Description	Additional Comments
Collecting Activity Table	Required	One line should be completed for each species in each location. The state, county, season or months of collection, and numbers for each type of collection should also be specified. In addition, information should include whether individuals will be adult males, adult females, juveniles, nestlings, and/or eggs.	It is strongly recommended to include a paragraph explaining the information in the Collecting Activity Table and justification of numbers, age classes, locations, and time of year.
Proposal and justification	Required	The proposal should include the following items: Justification for the proposed research (background, statement of problem, and hypotheses) Study site selection and description Species to be studied Time period of collection Field and laboratory methods Expertise of researchers to be conducting this research Literature cited	This section should be several pages long. Investigators may copy and paste from an existing grant proposal or may write a brief summary and include an entire grant proposal as a supporting document. Regardless, a Collecting Activity Table is still required. Statistical justification for the numbers of individuals requested is strongly encouraged. Additional specific justification must be submitted to collect birds on the most recent Birds of Conservation Concern List, which is available at http://www.fws.gov/migratorybirds/.
Description of background and expertise of researchers in conducting the proposed activities	Required	If this information is already contained in the investigator's permit file in the Migratory Bird Permit Office, this component is not necessary.	A curriculum vitae is helpful; however, a paragraph highlighting the investigator's expertise in the requested field of scientific work and field/laboratory methods also is necessary.
Justification for number of birds requested	Optional	It is preferable that this be a statistical justification such as a power analysis demonstrating the sample size needed for statistical significance.	This is especially important for bird species of conservation concern if the investigator is lethally taking individual birds, trapping and holding individual birds for longer than 2–3 hours, or requesting a large number of individual birds for trap and release.

Table 2. (cont.)

Component	Required or Optional	Description	Additional Comments
Explanation of population level effects	Optional	This information should detail the population-level effects (i.e., the proportion of the local population to be removed and citation[s] supporting the population estimate) and how the researcher proposes to avoid/minimize impacts.	Examples of minimization measures include the following: Utilize existing museum specimens Collect dead specimens from wildlife rehabilitation centers Distribute lethal-take of birds geographically and temporally Collect specimens outside the breeding season or toward the end of the breeding season when young of the year are independent Avoid lethal take of breeding birds Utilize blood rather than tissue for genetic analysis Coordinate project with other research projects collecting the same or similar species to share samples
Explanation of conservation benefit	Optional	This explanation would include information regarding the conservation benefits of the proposed project.	The explanation should indicate that collected specimens would be deposited in a museum. Working with local researchers will facilitate identification of those most suitable and willing to receive materials.

in question. All of these efforts will help minimize bird handling time, difficulties with identification in the field, and ensure proper source metadata pertaining to collected samples.

If a BBL permittee is uncertain of a bird's identification, guidance from the BBL is not to band the bird. The metadata for the sample(s) should reflect the uncertainty of the species, sex, and/or age of the bird. If a BBL permittee is confident of the species identification but not the bird's age and/or sex, multiple photographs of the bird need to be taken to record all key morphologic features. Important characteristics to photographically document include spread wing feathers and tail feathers, beak morphology, and general plumage. The BBL permittee should then consult with experienced hummingbird banders to reach a consensus. For US and Canadian BBL permittees (master and sub-permittees), there is a listserv (Humband) available for requesting expert opinions for challenging identification cases.

Some biologists use auditory traits to help identify hummingbird species (Clark and Mistick 2018b; Clark et al. 2018b), especially for male birds. However, it is important to ensure that the auditory traits are directly associated with the bird being captured, identified, and sampled. The Merlin Bird ID mobile device app (Cornell Lab of Ornithology; https://merlin.allaboutbirds.org/download/) is a useful resource but does not contain all hummingbird vocalizations or sonations (non-vocal communicative sounds made from wing or tail feathers; Feo and Clark 2010). Xeno-canto (https://www.xeno-canto.org/), a website dedicated to sharing bird sounds collected worldwide, is a useful resource for recordings of vocalizations from temperate and neotropical hummingbirds. Another useful reference for North American species is *Peterson's Field Guide to Bird Sounds of Western North America* (Pieplow 2019).

2.2. Safe Practices for Capturing Hummingbirds

A variety of traps have been used to capture hummingbirds. *The North American Bander's Manual for Banding Hummingbirds* (Russell and Russell 2019) describes several types of traps. The use of traps to safely capture birds depends on the individual's experience and skill level. When trapping, certain precautions must

be taken to ensure the safety not only of hummingbirds but also of researchers and bystanders. Drop-curtain and drop-door traps require researchers to sit remotely while the curtain or trap door is typically controlled by a monofilament line. This line should be clearly marked with a material, such as vinyl flagging tape, to prevent tripping or other hazardous conditions, especially when working in a public location. When using traps, the best practice is to wait until birds are feeding or at least perched on the feeder within the trap before closing the trap door or dropping the net. If a trap is set properly (i.e., the trap is not raised too high and/or the feeder is placed as far as possible from the door), chances of harming hummingbirds are minimized when triggering the trap or closing the door. Regardless of safe-trapping practices, to further minimize bird injury, all components of the trap should be lightweight and the trap curtain or door should not close with excessive force.

When capturing and processing hummingbirds, researchers need to consider the time of day, temperature, wind speed, precipitation, and the number of trained individuals available to extract and process birds. In order to minimize situations that predispose birds to hypoglycemia, hypothermia, and respiratory and/or cardiac distress and/or arrest, researchers need to establish written protocols defining conditions during which netting and trapping should be avoided or curtailed. Every set of circumstances is unique; therefore, a bird's condition must be constantly monitored and activities reduced or aborted as necessary.

Special precautions should be taken when trapping or mist netting and sampling at first light, because some hummingbirds may be ending a prolonged fast and may require time to re-establish energy reserves. Similarly, care should be taken prior to sunset when some hummingbirds will be building up their fuel resources prior to entering torpor. Disturbance prior to sunset could negatively impact a bird's ability to survive through the night. This is especially important during winter months and in geographic locations where hummingbirds face challenging weather conditions. From a welfare perspective, one universal guideline is that hummingbirds should not be captured or processed within 30 min after sunrise or before sunset. This time guidance might need to be extended depending on the situation.

In general, a researcher can consider a bird caught at a feeder to be seeking food and the bird might have had a small meal immediately prior to capture. In contrast, if a bird is caught in a mist net, there is no way to know when the bird last ate; therefore, the researcher should assume the bird could be close to an energy crisis. During the breeding season, hummingbirds may be caring for dependent young and have high energy needs; therefore, birds should be extracted from traps and netting and processed as quickly as possible. The BBL does not have specific guidelines for checking mist nets, but under most circumstances the authors and B. Peterjohn (pers. comm., 28 June 2020) recommend checking at least every 15 min. However, in temperatures above 27° C (80° F) or below 10° C (50° F), mist net checks are recommended every 10 min or more often. To ensure bird safety, regular monitoring protocols should be developed for all trapping activities, even when traps are open. Open drop-net traps can be problematic because even though the trap is "open," hummingbirds might fly to the top of the trap and not find an escape route. Capture stations must be prepared to treat birds compromised by health issues or a hypoglycemic/hypothermic event, which includes providing supplemental heat and small volumes of sugar water.

Research protocols need to describe how many feeders to cover and/or remove to concentrate hummingbirds at a trapping station. When setting up a hummingbird capture station, researchers should assess food sources available within a 1.6–3.2 km radius of the research site. For isolated feeding stations (i.e., those with no other feeders available within 3.2 km) at a time of year when flowers and insects are scarce (e.g., California during the dry season), decreased energy sources and increased competition at remaining feeders can result in birds becoming exhausted. In such conditions, the trapping station should be operated for shorter periods of time (approximately 20–40 min), after which the original number of feeders should be returned to their original hanging locations or uncovered. This protocol can be followed intermittently as needed. Researchers should estimate time intervals for returning the feeders by closely observing feeding activity and the status of birds at the banding table. The less food availability, the shorter the continuous trapping time should be. For circumstances where each

bird is being held for an extended period of time (e.g., for blood and feather sampling and/or tag placement), feeders should be returned to their hanging locations and/or uncovered while each bird is being processed and then removed to recapture the next bird.

In situations where additional feeders surround the research site (i.e., within a 1.6–3.2 km radius from the trapping location) and/or natural foods are plentiful, hummingbirds will go elsewhere to feed so that reducing the number of feeders to facilitate trapping should not have a negative impact. For locations hosting large numbers of hummingbirds (i.e., >100 birds), one to two uncovered feeders can be left hanging in addition to the feeder(s) at the trapping station(s). Because competition at the uncovered feeders can be relatively intense, some hummingbirds may look for feeders at other locations; thus, fewer birds will be available to enter the traps. If this scenario involving plentiful feeders applies to a private residence research location, property owners need to be reassured that birds will not starve if the majority of feeders are removed/covered for approximately 20 min.

Acceptable weather conditions for capturing hummingbirds will depend on capture techniques. In general, netting and trapping should be avoided during inclement weather (especially involving precipitation) or high wind conditions because such conditions can compromise a bird's ability to thermoregulate and will increase energy demands. The range of cold temperatures conducive to working with hummingbirds will depend on other variables, such as humidity and wind. Banding is possible during colder temperatures (-4° C [25° F] to 4° C [39° F]) (B. Peterjohn pers. comm., 28 June 2020) if the bird is removed immediately from the trap and processed within 2–4 min of being captured. Banding also can occur when temperatures are below 0° C (32° F) if birds are brought into a warmer environment for a short period of time. Capturing and sampling hummingbirds during extreme heat also can be problematic. Once captured, birds must be processed quickly to avoid overheating. If captured birds are brought indoors for processing, investigators should work only in rooms without ceiling fans and with ceilings low enough to allow escaped birds to be captured with a net. A long-handled net is a necessary piece of equipment when working indoors. The net should be similar in weight and material to a butterfly net to avoid crushing the bird when capturing it. Prior to initiating work indoors, covering windows and glass doors should be considered to prevent an escaped bird from being injured. Some investigators prefer uncovered windows or glass doors to facilitate capture as the birds are attracted to them as a possible escape route. Whether to cover or not cover windows and doors depends on the acceleration speed that a bird could attain before hitting the structure. Opening a door that leads to the outside might help with guiding a bird outdoors if the indoor ceilings are not too high.

2.3. Handling, Restraining, and Retaining Hummingbirds

With all captured hummingbirds, the researcher needs to minimize detaining and handling time; thus, being efficient is essential. Individuals who have not handled hummingbirds previously can initially learn bird handling skills and gain confidence using captive birds of similar size and weight (e.g., zebra finches). Another way of obtaining experience is to volunteer and train at a passerine banding station. At the time this manuscript was written, the general policy of the BBL was that handling several hundred passerines under the supervision of an experienced bander was required before a request for a hummingbird banding permit would be considered (B. Peterjohn, pers. comm., 28 June 2020).

Obtaining samples from hummingbirds requires the handler to pay attention to the sampling task at hand while simultaneously monitoring the bird's condition. When restraining hummingbirds, handlers should monitor birds for signs of compromise or stress. Compromised hummingbirds may continually vocalize, gape, repeatedly close their eyes, maintain their feathers in an erect position, or spontaneously shed large numbers of contour (body) or tail feathers. Birds experiencing an energy crisis may appear to fall asleep or exhibit minimal response to stimulation. If these conditions occur, the bird should immediately be placed in a bird bag or released, depending on the researcher's experience level. When in doubt, the researcher should err on the side of caution and release the bird. If the bird is not released, restraint can be attempted again after the bird has rested, but this should be done by the person on the

team with the most handling experience. Depending on the bird's condition, offering sugar water or warming the bird prior to release (if hypothermia is of concern) may be helpful. If a bird shows signs of having an energy crisis during processing—and to minimize the risk of this happening to other birds—feeders should be restored to their original numbers and locations for a period of time so that the remaining population of hummingbirds can replenish their energy stores.

The most critical thing to be aware of when restraining any bird is to avoid putting pressure on the thoracic region, which will compromise the animal's respiration. Birds do not have a diaphragm; instead, they use their intercostal muscles to move the keel up and down, which imitates a bellows and moves air in and out of the respiratory tract (i.e., air sacs, airways, and lungs). Pressure on the keel can prevent the chest area from rising and falling and can result in suffocation. Therefore, proper restraint entails holding a bird with gentle pressure applied at its sides and not from front to back.

When handling a hummingbird, it also is important to keep the bird upright and not allow the head to go below the plane of the tail. This positioning will prevent fluid in the abdominal region from flooding the lungs if the bird has liver disease. For female birds, upright positioning will minimize air sac compression by reproductive organs or a developing egg. Avoiding pressure in the crop region will minimize regurgitation and aspiration (passage of ingesta into the respiratory tract) of sugar water, especially if the bird was caught at a feeder. If a bird starts to regurgitate fluid, it should be immediately placed on a flat surface, restraint should cease, and the bird allowed to clear the fluid itself. In severe cases, the bird might need to be placed in a field intensive care chamber (see section 2.5) to recover. The handler should never attempt to clear fluid or turn the bird upside down to let the fluid drain; a conscious, unrestrained, and normally positioned hummingbird will be better able to clear the fluid on its own.

Several methods for handling and restraining hummingbirds have been described previously (Russell and Russell 2019), and best safe practices will depend on the handler's skill level and hand conformation and size. Depending on the handler's hand confirmation and size, it is possible to handle larger hummingbird species using published small passerine restraint techniques (Russell and Russell 2019). For smaller-sized hummingbird species (less than 6 g), the hummingbird bander's hold can be used where the bird is restrained in one hand, with hyperflexion of the third finger into an upside down 'V' shape which creates a crevice that allows for cradling the bird's head and neck (Fig. 1 A and B), and the remaining fingers cup the bird's body. The hummingbird bander's hold decreases the chance for cervical dislocation that can occur with the "traditional" bander's grip used for passerines. With the "traditional" bander's grip used for passerines, the second and third fingers are extended (straightened) and used to restrain the head. However, if this grip is used with small-sized hummingbirds (less than 6 g), excessive head and neck traction can result in cervical dislocation. Using the hummingbird bander's hold, where the third finger is bent (hyperflexed), the handler can restrain the head without too much traction, visually/tactilely monitor the bird's respiratory/activity status, observe the eyes for closure, and minimally compress the chest region while creating an environment where the bird feels enclosed and is less likely to struggle. During restraint, the bird is positioned upright. The bird's safety and the optimal technique depends on the handler's hand size, finger conformation, finger dexterity (i.e., how much the handler can hyperflex the third finger), and positioning of other fingers relative to the base of the hand for cupping the bird. To maximize dexterity, prospective handlers should practice third finger hyperflexion without a bird in hand.

For containment and transport, hummingbirds can be placed in a soft, well-ventilated bag that prevents excessive movement, which can deplete a bird's energy stores. Instructions for making safe, effective restraint bags from seine material are found in the *North American Hummingbird Banders Manual* (Russell and Russell 2019). Holes in the seine mesh should be large enough to provide adequate air flow but sufficiently small to avoid catching the tips of a bird's primary feathers, which can result in shoulder dislocation or damage to flight feathers. Using the seine material bag, the researcher can see and position a bird to gain access to a leg for banding or toenail for blood sampling (see section 4.2).

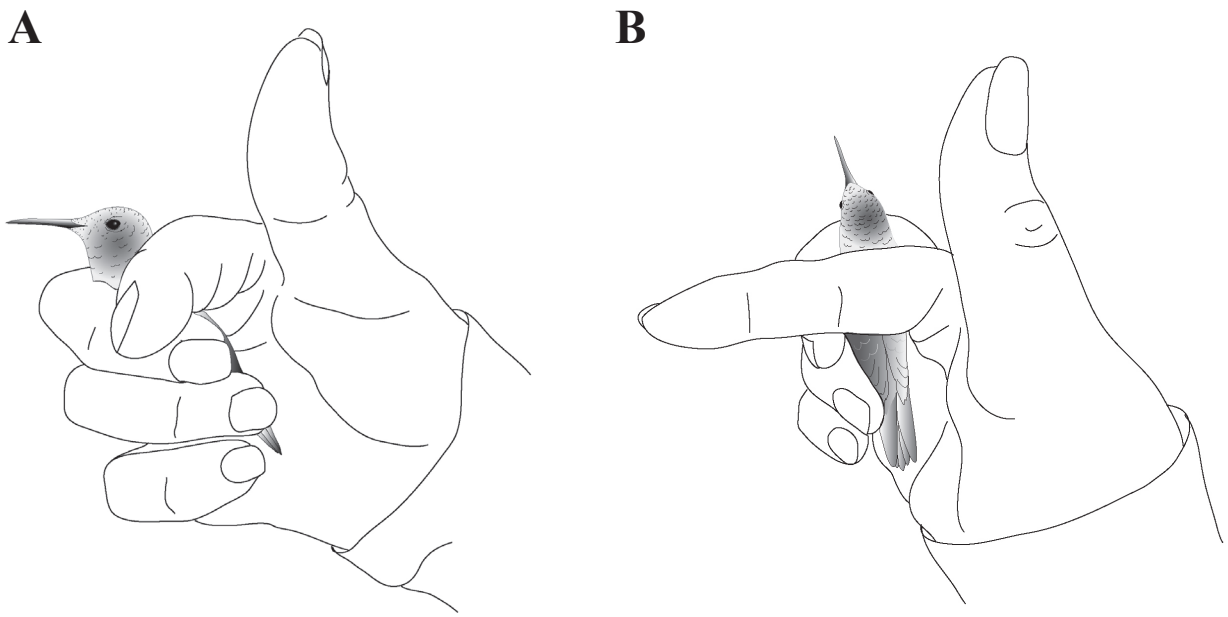

Figure 1. llustration of lateral (A) and dorsal (B) views of the hummingbird bander's hold. Note that the third finger is bent into a distinct "V" shape and the index finger is slightly flexed and positioned behind the bird's head, while the fourth and fifth fingers are curled into the base of the hand. The bird is contained in a "cupped hand", avoiding front to back pressure, so it can breathe. Hyperflexion (bending) of the third finger cradles the head and reduces the chance for cervical dislocation. The safety of using this restraint technique will depend on the handler's finger dexterity and confirmation and hand size.

Weather permitting, hummingbirds can be contained in a bird bag. Best practice is to offer all birds a limited meal before they are placed in a bird bag; however, they should not be allowed to overfeed. Depending on the situation (e.g., whether or not a bird drank sugar water prior to being placed in the bag, the amount of sugar water consumed, the ambient temperature, and the amount of movement in the bag), birds should be offered sugar water every 15–45 min to prevent hypoglycemia. Bags containing hummingbirds should be placed in a safe, shaded location to prevent birds from becoming overheated, and the bags should always be suspended and not laid on flat surfaces, including tables and floors. A bird in a bird bag should never be placed on the ground. In addition to avoiding inadvertent harm, hanging bird bags allows for visually monitoring birds between capture and processing. Various stands are used for hanging hummingbirds contained in holding bags. For stations where sampling is the major focus (as opposed to banding/capture/recapture), a small stand made of polyvinyl chloride (PVC) pipe and fittings can be used (Fig. 2). The bag must be hung high enough so that the bird is suspended and not allowed to come in contact with a flat surface where it will struggle and potentially harm itself or damage its feathers. The damage that a struggling bird on a flat surface might inflict on itself may not be visible, but continuous struggle against a firm surface can result in muscle injury. The authors do not hang bags by the drawstring (especially when using short stands) but rather use the holes in the seine bag to hang on the hook.

If a researcher needs to retain a hummingbird in captivity for an extended period of time (i.e., >30 min) for sampling purposes, the bird can be placed in a cage with food available *ad libitum* (Russell and Russell 2019). Similar to recommendations for holding birds in bags, enclosures should be placed in a shaded area with no direct sunlight to prevent overheating and should be visible by a team member at all times. Commercial butterfly enclosures with mesh on at least one side afford good ventilation and offer one option for retaining birds temporarily. This is particularly

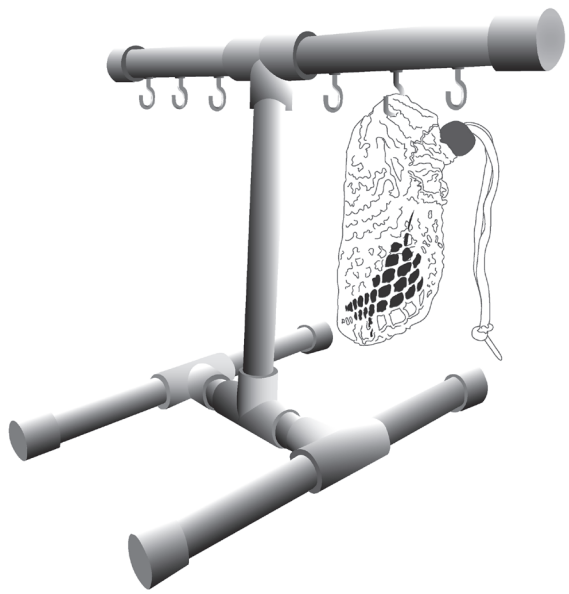

Figure 2. Illustration of a bird bag holding stand made of polyvinyl chloride pipe and fittings. This stand can be used for suspending hummingbirds contained in holding bags at the field work-station until processed. Note that a hole in the seine mesh (as opposed to the cord of the drawstring) is used for suspending the bag from the hook so that the entire holding bag is suspended.

helpful if the research activity requires that the bird not be in a bird holding bag or if the bird needs to be acclimated to captivity for a captive study. However, butterfly cages are not a replacement for bird bags, because birds will fly against the sides of the cage and damage their feathers or continuously fly and become exhausted. Larger enclosures can be made with PVC tubing and small-gauge netting. When caged, a bird must be observed drinking from the offered food source before it is left unobserved for any extended period. If the bird is not seen drinking, it should be caught and fed every 20–30 min until it learns to drink from the food source.

When providing food to a hummingbird that may be kept in captivity for an extended period, using syringes instead of an outdoor hummingbird feeder is recommended. Syringes are easy to fill and can be disassembled for cleaning. In addition, volume markings on syringes allow for tracking food consumption and controlling the amount of hand feeding. Syringes with a 15 mL or 30 mL capacity (e.g., Becton Dickinsons oral syringes; https://www.bd.com/en-ca/offerings/capabilities/syringes-and-needles/oral-and-enteral-syringes/oral-syringes) are recommended. The syringe can be suspended in the cage by a flexible wire, which is coiled around the syringe and extended up to form a hook by which the syringe is secured to the top or side of the cage. The syringe opening should be pointed at an angle that allows the bird to easily gain access to the food while preventing the sugar water from leaking out of the syringe, as might happen if the opening were pointed straight down. Hummingbirds should be monitored regularly to ensure that they learn how to drink from the syringes. To that end, multiple syringes should be made available to a newly captive bird to improve its chance of trying one of them. A perch can also be placed close to one of the syringes to facilitate access. If a bird is not observed feeding, then it should be captured and its beak placed into the syringe opening. This procedure may be necessary multiple times until the bird is observed feeding on its own. All syringe feeders should be cleaned with an appropriate cleaning solution between each use and changed daily. For long-term housing of birds, an enhanced diet is recommended instead of sugar solution alone. Nekton-Nektar-Plus® nectar concentrate (Hoheneichstraße, Germany) can be used for adult hummingbirds. The manufacturer recommends making new formula twice a day (morning and afternoon) because it spoils easily in warm environments. The volume of food offered should meet the energetic and nutritional demands of the species, age, and sex of the captive bird being held. For juvenile birds, additional factors should be considered in conjunction with veterinary advice.

Hummingbirds can be transported in bird bags either by hand or in a car. As previously described, the amount of time hummingbirds are held in bird bags should be limited, the researcher must ensure that the bird has used a feeding system before transport, and access to a stable food delivery system throughout transport is imperative. If a bird is transferred in a car, the bird bag must be suspended to avoid inadvertent injuries. Hummingbirds should not be left unattended in cars in hot environments, and air conditioning should be used to prevent overheating.

2.4. Clinical Warning Signs When Handling and Sampling Live Hummingbirds

An understanding of hummingbird anatomy and physiology is critical to ensuring welfare during handling. For general guidelines, see the *North American Bander's Manual for Banding Hummingbirds* (Russell and Russell 2019). More specific advice regarding threats facing hummingbirds during handling, including organ system specific issues and conditions of specific concern, is provided below. Figures 3 and 4 illustrate a hummingbird's internal anatomy.

2.4.1. Respiratory system.—A hummingbird gaping or panting during handling usually indicates that the bird's breathing is impaired. Gaping can be a result of excess pressure being applied to the chest region, which impedes movement of the bird's keel and restricts gas exchange and oxygen availability. Gaping or panting is an emergency situation that can result in hypoxia, cardiac arrest, and death within as little as 5 sec after the onset of the clinical signs. Once a bird starts gaping or panting, the handler must loosen their grip to relieve compression on the chest region and release the bird into a holding bag, a container, or the wild. Airway compromise also can result from regurgitated fluid that is aspirated. Other reasons for gaping include air sac compression by enlarged organs, fat deposition, and/or an egg. To reduce the incidence of respiratory distress during sampling, birds should be maintained in a head-up position with just enough restraint to prevent escape, and handling time should be minimized.

2.4.2. Reproductive system.—During breeding season, female birds (hens) must be handled with care. At the onset of handling, the abdominal area should be assessed for the presence of an egg. Enlargement of the reproductive tract displaces other organs and can compress the air sacs. When evaluating whether a female bird is gravid, the bird should be restrained in an upright position to minimize pressure of an egg on the air sacs and lungs and the birds should be processed quickly. Gravid birds or birds that appear to have laid an egg recently (i.e., those with an extremely enlarged and swollen cloaca) should be released and not sampled to minimize the time the hen is away from the nest.

2.4.3. Torpor.—Many researchers who have worked with hummingbirds are familiar with torpor, a state of decreased metabolic activity, in which all non-essential functions are reduced and body temperature may drop to below 10° C (50º F) in North American species (McKechnie and Lovegrove 2002) and as low as 3º C (37º F) in Andean species (Wolf et al. 2020). Torpor is a mechanism for hummingbirds to conserve energy throughout the night (Ruf and Geiser 2015). Along with a reduction in body temperature, torpor is characterized by elevated feathers and lack of a response to movement or touch. Many hummingbirds go into torpor when energetically stressed during emergencies, often caused by low food availability or sudden change in environmental conditions (Hainsworth et al. 1977). Increasing evidence suggests that several hummingbird species use torpor regularly, even when they have high energy reserves (Krüger et al. 1982). However, this distinction may depend on the size of the species, with smaller species employing torpor more often than larger ones (Shankar et al. 2020b). This is an important factor to consider when working with smaller species, because smaller hummingbirds may go into torpor despite having food available *ad libidum* (Krüger et al. 1982), while larger species may use torpor less but employ it during times of duress (Hainsworth et al. 1977). When birds are coming out of torpor, they will respond to touch and stimulation, stretch their wings (waking behavior), clamp their feet reflexively, and vocalize with a squeaking pitch. This "keening note" is a unique sound, similar to one a mouse might make, and not one observed in active hummingbirds except during extreme distress (Stiles 1982). Although torpor itself is not a life-threatening condition, special consideration should be given to these events relative to sampling. Because hummingbirds might undergo torpor at night and come out of torpor early in the morning, researchers should never take blood samples from hummingbirds for at least 30 min after sunrise or 30 min prior to dusk.

2.4.4. Hypoglycemia and other compromised conditions.—Although hummingbirds can use torpor to conserve energy, hummingbirds in a torpor state predominately have been documented at night (Calder 1994; Ruf and Geiser 2015). In general, a healthy

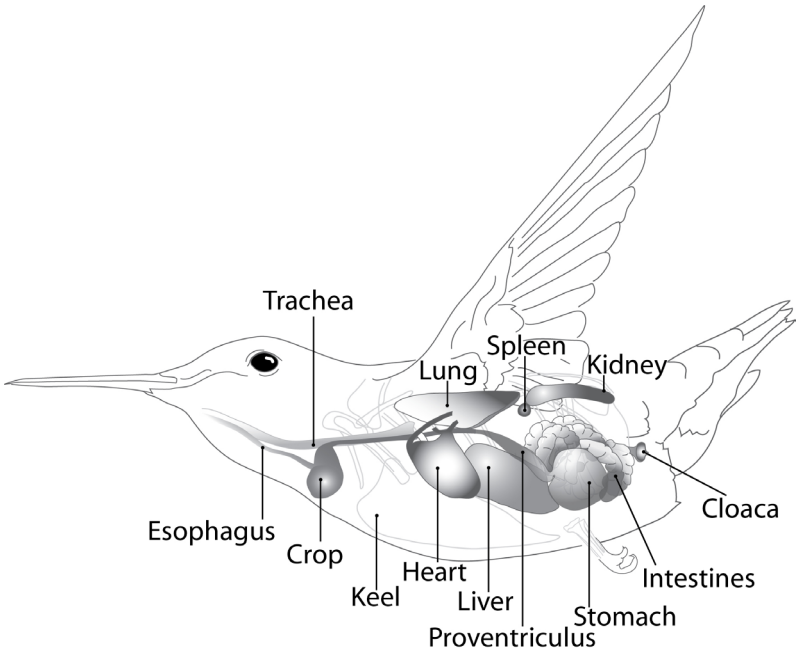

Figure 3. Anatomic drawing (lateral view) of soft tissue organs in the coelom of a hummingbird.

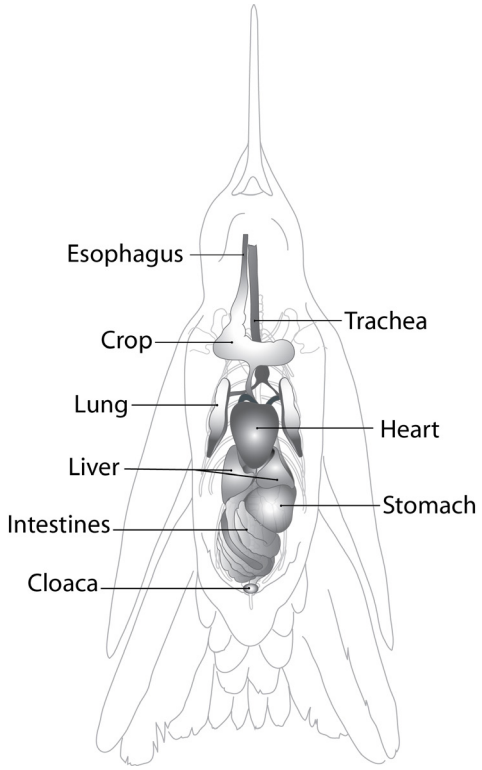

Figure 4. Anatomic drawing (ventro-dorsal view) of soft tissue organs in the coelom of a hummingbird.

hummingbird should not go into torpor during the day and in response to handling and sampling. Therefore, a bird that appears to be going into torpor during handling, restraint, banding, or sampling during the day is more likely to be hypoglycemic or hypothermic or experiencing cardiac/respiratory arrest.

Metabolic requirements of hummingbirds limit how long they can go without feeding; therefore, the time between capture, sampling, and feeding must be carefully monitored. Birds that become hypoglycemic, hypothermic, or are entering another compromised state may exhibit several warning signs, including erect feathers and dull and/or drooping eyes, which can gradually progress to overall unresponsiveness. Thus, it is important to continuously monitor birds for these signs during handling and especially after blood sampling, because the bird's status can deteriorate rapidly. If a bird begins to show any of these signs, processing should be immediately discontinued and the bird's condition assessed. If the bird is conscious, sugar water should be offered. A bird that is extremely hypoglycemic must be fed sugar water if it is going to recover. In rare cases, the researcher might have to carefully open the bird's beak and deposit a tiny drop (approximately 2 µl) of concentrated sugar water on the tip of the tongue, which can be done using tuberculin or insulin syringes with the needle removed. An eye dropper can be used as a last resort but this technique risks spilling sugar water on the bird's feathers. If the bird responds by extending and withdrawing its tongue several times, it is ingesting the sugar water. If the bird does move its tongue, the researcher should wait 2 min and repeat the process. Hypoglycemic hummingbirds can recover very rapidly and voluntarily ingest large volumes of sugar water once their blood sugar concentrations are restored. If the bird recovers, it may be appropriate to resume data collection. If the bird does not feed or feeds but does not recover, it should be placed in a field intensive care chamber (see the following section), provided supportive care (i.e., supplemental heat and a quiet environment), and monitored closely.

2.5. On-site Care of Hummingbirds

When working with hummingbirds, it is best practice to have an intensive care chamber and supplemental feeding sources available at study sites. Some biologists place a compromised hummingbird inside their clothing and use their own body heat to provide the necessary supplemental heat. However, depending on an individual's clothing (e.g., the material and number of cloth layers), this practice could reduce ventilation and expose the bird to an accumulation of carbon dioxide. In addition, the bird could be inadvertently crushed. Placing a compromised hummingbird that has been fed a sugar meal in an intensive care chamber allows the bird to recover by minimizing exertion that might occur with continued handling. The intensive care chamber can be made from a small container with air holes. A fisherman's bait bucket, which is typically made of sturdy plastic and contains air holes in the lid as well as a trap door for easy access to the inside of the container, can be used for this purpose. A heat source that does not emit fumes can be placed inside the intensive care chamber; one recommendation is a re-useable hand warmer (HotSnapZ®, La Porte, Indiana), which uses sodium acetate crystallization to generate exothermic heat. A tightly woven cloth that will minimize the risk of a bird catching its nails can then be placed between the bird and heat source. A small tuberculin or insulin syringe without a needle can be used to offer sugar water or a balanced electrolyte solution to help restore energy reserves. As mentioned previously, an eye dropper or a plastic transfer pipette is not ideal because it risks spilling sugar water on the bird's feathers.

3. MARKING HUMMINGBIRDS

3.1. Trends and Applications

A literature review revealed 46 studies that used some form of marking to address a specific research objective. Of these, the majority used leg banding (16) followed by color marking (19), colored acetate plastic tags on the leg or back (3), radio-frequency identification tags (6), radio-telemetry tags (7), and plastic colored bands (1). Table 3 summarizes these studies. Note that for any marking methods discussed below,

Table 3. Summary of methods used to mark hummingbirds as reported in published studies.[a]

Method	Count	Permanence	Applications	References
Banding	16	Years	Population biology, conservation, ecology	Calder III et al. 1983; Miller and Gass 1985; Inouye and McGuire 1991; Mulvihill et al. 1992; Oniki 1996; Hilton Jr. and Miller 2003; Wethington and Russell 2003; Bassett and Cubie 2009; Temeles et al. 2009, 2013; Hurly et al. 2010; Cubie 2014; Maglianesi et al. 2014b; Supp et al. 2015; Zenzal and Moore 2016, 2019
Acetate plastic colored tags on back, leg or leg band	3	Weeks to years	Population biology, behavior ecology	Stiles and Wolf 1973; Waser and Calder 1975; Kapoor 2012
Plastic colored bands	1	Years	Ecology, behavior	Temeles and Bishop 2019
Color	19	Days to months	Behavior, population biology, conservation, ecology	Wolf 1969; Baltosser 1978; Ewald and Carpenter 1978; Goldsmith and Goldsmith 1979; Stiles and Wolf 1979; Trombulak 1983; Gill 1988; Stiles 1992; Hilton Jr. and Miller 2003; Temeles et al. 2006; Clark and Feo 2008, 2010; González and Ornelas 2009; Feo and Clark 2010; Hurly et al. 2010; Clark 2011b, 2014; Goloff and Burch 2012; Zenzal and Moore 2016; Tello-Ramos et al. 2019
RFID	6	Days (glue application) to years (subcutaneous injection)	Behavior, population biology, conservation, ecology	Brewer et al. 2011; Hou et al. 2015; Ibarra et al. 2015; Zenzal and Moore 2016, 2019; Bandivadekar et al. 2018
Telemetry	7	Days	Behavior, conservation, ecology	Hadley and Betts 2009; Zenzal et al. 2014, 2018; Zenzal and Moore 2016; Hazlehurst and Karubian 2018; Céspedes et al. 2019; Pavan et al. 2020

[a] Literature searched until September 2020.

federal and state permits are required. Depending on the research objectives and corresponding biological specimen sampling plan, it may be necessary to mark hummingbirds to avoid resampling a bird on the same day and location or re-sampling a bird if the recapture interval is short. When marking hummingbirds, it is important to consider the long-term effects on the birds and to use methods that will have the least impact. If long-term population monitoring and/or resampling individual birds is not necessary, a minimally invasive method, such as subtle paint marking, may be the best option. However, the impact of various marking methods must be considered carefully. For example, during the breeding season, paint can compromise the cryptic appearance of a female on the nest, or clipping certain feathers might impair the sounds that a male bird's feathers make during courtship dives.

3.2. Feather Clipping

Feather clipping is an easy way to mark a bird without banding and to ensure identification of recaptured birds that were sampled. In past studies, 2–3 mm of the feather tip was clipped, or small V-shaped cuts were made in the tail feathers or the distal portion of secondary wing feathers. Cutting primary wing feathers hinders flight and, for some species, might impact a bird's ability to make sounds; therefore, it may be more appropriate to clip other feathers for marking purposes. Scientific studies have shown that hummingbird tail

feathers are important during courtship and for communication (Clark et al. 2013a, 2013b, 2018a; Clark and Mistick 2018a). For example, tail feathers R4 and R5 (Fig. 5) play an important courtship role in males of some hummingbird species, so these feathers should not be clipped during breeding season. Hummingbirds in the bee clade make sounds with their tail feathers (Clark and Mistick 2018b), so clipping secondary wing feathers is a more acceptable alternative. However, there are exceptions even to clipping secondary feathers, such as in the *Archilochus* species whose primary/outer secondary feathers have been modified to produce sounds. Whichever feather is clipped, one must be aware that it will remain unchanged until the following molt, the timing of which varies by species and location.

3.3. Dye, Pigment, Paint, and Miscellaneous Marking

A variety of dyes, pigments, or paints have been used to identify previously marked birds (in which case a single uniform mark can be used) or individual birds from a distance (by using a unique shape, orientation, or color combination). This approach is ideal when an investigator wants to avoid distressing individual birds that have already been captured, or when the goal is to observe individual behaviors of a species with predictable, small-scale spatial movements (e.g., territorial or lekking hummingbirds). An advantage of paint marking is that it can be used in conjunction with trap cameras or videos because the markings are

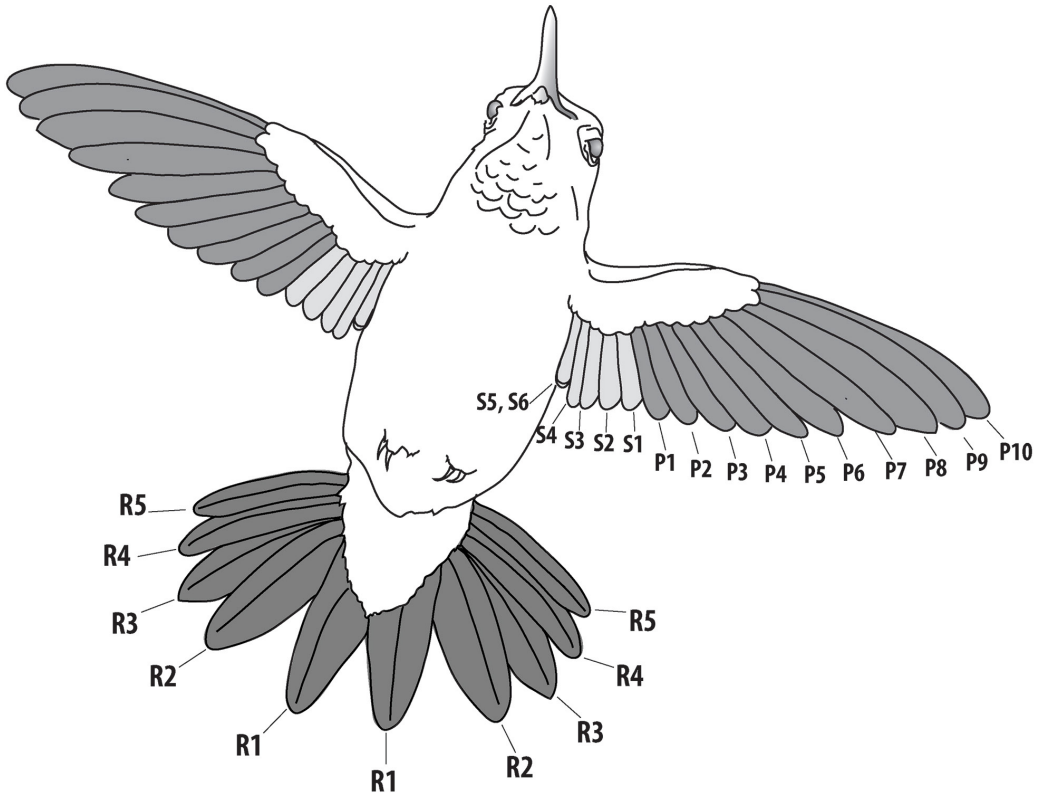

Figure 5. Illustration of extended wing (numbered wing feathers; remiges) and flared tail (numbered tail feathers; rectrices) feathers of a hummingbird in dorsal recumbency.

readily visible. Animal detection software, such as Motion Meerkat (Weinstein 2015), is available for scanning video footage for visitations and movement patterns of individual hummingbirds. Motion Meerkat also allows investigators to easily delete video footage that does not contain hummingbirds.

Published paint marking studies have reported the use of several body regions, including the crown (Temeles et al. 2006), the neck or chest (Hilton, Jr. and Miller 2003; Hurly et al. 2010), and the back (Stiles 1992). The North American Banding Council recommends using the crown for marking birds and this site is considered the best practice for hummingbirds (Russell and Russell 2019).

Although a range of pigments have been used and reported in the literature, non-toxic, water-based, fabric paint is generally accepted as best practice and can remain on a bird for weeks to several months (Russell and Russell 2019). The potential impacts of using dyes, pigments, or paint to mark a bird always should be considered prior to application. It is crucial to minimize the amount of material that is applied. Primary harmful effects include ingestion, toxicity, and increased visibility that can result in aggression or predation. Secondary effects include reduced reproductive success, reduction in feather waterproofing, and inability to erect feathers completely when a bird is trying to maximize insulation.

3.4. Banding (Color and Aluminum Bands)

Banding remains the primary means for tracking hummingbird movements and longevity as well as for gathering other important demographic data. The most common banding method utilizes aluminum bands. However, one study (Temeles et al. 2013) reported identification of individual hummingbirds at a distance using specialized colored Darvic tarsal bands (Avinet Research supplies®, Portland, Maine). A major constraint when using color bands for marking hummingbirds is the limited visibility of bands afforded by the extremely small hummingbird tarsi that are covered with feathers. Because of a hummingbird's small size, color banding is typically limited to a single colored metal band. For a detailed guide on how to band hummingbirds, see the *North American Banders' Manual for Banding Hummingbirds* (Russell and Russell 2019).

For any banding event, particularly if recapture is not highly likely, the investigator might consider whether banding is necessary. Anecdotal experiences suggest regional and species-specific leg swelling patterns in breeding female hummingbirds. Suspected band-associated leg injuries were reported in recaptured female Anna's Hummingbirds (*Calypte anna*) in California during the breeding season (Colwell 2011). Until further studies are performed, researchers should consider possible seasonal changes and the importance of determining proper band sizes for breeding females. Another consideration when banding hummingbirds is the relatively high prevalence of pox lesions in the metatarsal region. Banding a hummingbird with pox lesions on any area of the body is not advised as the lesions might progress to the metatarsal region, thus effectively tightening the band and restricting blood flow to the distal limb.

3.5. Acetate Plastic Colored Tags

A few hummingbird studies have used tags made from acetate plastic colored sheets (Stiles and Wolf 1973; Waser and Calder 1975) or ethylene vinyl acetate (EVA) plastic colored beads (Perler™ beads, Igdesigngroup, Atlanta, GA) (Kapoor 2012). To mark individual hummingbirds, tags were attached to the back, leg, and/or leg band (Stiles and Wolf 1973; Waser and Calder 1975; Kapoor 2012). Acetate tags have been reported to be effective for marking hummingbirds (Stiles and Wolf 1973; Kapoor 2012); however, there are concerns regarding their safety. One investigator used back tags constructed of fused color plastic beads for identifying birds as an alternative to colored plastic leg bands (Kapoor 2012). While this method offers the advantage of short-term visual detection of marked birds, it may have limitations, especially in the case of breeding females who might draw predators to nests. One report (Waser and Calder 1975) raised concerns that acetate tags may impair hummingbird nest construction, which could reduce breeding success. Anecdotal experiences also suggest hummingbird leg-related injuries associated with the use of acetate tags attached to either the leg or leg band. Stiles and Wolf (1973) warned that an acetate tag too tight around a hummingbird's leg will restrict circulation with eventual loss of the foot. Further studies are needed to evaluate the safety of acetate tags before they are used to mark hummingbirds.

3.6. Radio-frequency Identification/Passive Integrated Transponder Tags

Radio-frequency identification (RFID) technology is a method used to track hummingbird movement and presence (Brewer et al. 2011; Zenzal et al. 2014; Hou et al. 2015; Ibarra et al. 2015; Zenzal and Moore 2016, 2019; Bandivadekar et al. 2018). RFID tags have been miniaturized and do not require batteries; instead, they are powered by electromagnetic induction. In addition, RFID tracking antennas may be placed at hummingbird feeders or around flowers. When a tagged bird passes through or near the antenna, the unique identifying code is recorded along with the date and time, thus providing both temporal and geographical documentation. Tag events are limited to locations with antennas, and the maximum distance for a tag read depends on the type of hardware (i.e., tags and antennas) employed. There is an inverse relationship between tag detection sensitivity and reading distance; one study reported a 25.4–30.5 cm maximum tag reading distance from the center of a circular antenna (Bandivadekar et al. 2018).

A type of RFID tag called a passive integrated transponder is most commonly used for hummingbirds due to their small size and mass. Tags can either be glued to the feathers (with eyelash or surgical glue) on the back of a bird or injected under the skin (subcutaneously) (Brewer et al. 2011; Hou et al. 2015; Ibarra et al. 2015; Bandivadekar et al. 2018). If the tag is glued, extreme care must be taken to avoid inadvertent gluing of the primary feathers, thereby rendering the bird flightless. Using a viscous glue in small amounts helps control glue application and minimizes feather contamination. Most published studies report gluing or injecting RFID tags in the intrascapular region (i.e., between the shoulder blades) of hummingbirds (Ibarra et al. 2015; Zenzal and Moore 2016; Bandivadekar et al. 2018). However, when using subcutaneous application, the tag must be placed in the mid lumbar region to avoid rupturing the cervicocephalic air sacs and causing generalized subcutaneous emphysema (collection of air). If the tag is placed too far cranially, chances of cervicocephalic air sac invasion are substantially increased. After placing a tag subcutaneously, the entrance site can be closed with surgical glue or suture. In the authors' experience, suture used in a simple interrupted pattern achieves the best closure and avoids glue contamination of wing feathers. RFID tag placement on hummingbirds appears to be successful; however, there are only a few published studies reporting use of this technology (Brewer et al. 2011; Hou et al. 2015; Ibarra et al. 2015; Zenzal and Moore 2016; Bandivadekar et al. 2018) and more work is needed to determine best practices and long-term impacts.

3.7. Very High Frequency Radio Telemetry Tagging

In recent years, very high frequency (VHF) tags have been sufficiently miniaturized for use in some larger hummingbird species. Whereas RFID tags record information only at sensor locations, VHF tags allow for detailed study of individual movement. However, VHF tags require batteries and are therefore significantly larger and heavier than RFID tags. To meet the standards of the BBL guidelines on auxiliary marking, the total weight of any tag or any glued or "backpack" style attachment device must be less than 3% of the bird's body weight. From a welfare perspective, the lean (non-migratory) body weight should be used for calculations. Additionally, given their small size, the battery life of VHF tags generally is limited to a few days to two weeks.

Two published studies reported using eyelash glue and/or surgical glue to attach VHF tags to the intrascapular region of hummingbirds (Hadley and Betts 2009; Zenzal et al. 2018). As described with application of passive integrated transponders, extreme caution is necessary when using glue near wing feathers. It is also helpful to trim the tag antenna length shorter than the bird's wing length to preclude interference with flight and to decrease the risk of the bird removing the tag.

4. OBTAINING AND STORING BLOOD SAMPLES FROM HUMMINGBIRDS

4.1. Trends and Applications

A literature review identified 28 published manuscripts that specified blood collection from hummingbirds, including brachial, ulnar, jugular, or tarsal venipuncture (i.e., blood collection from a vein) and toenail clipping (Table 4). Toenail clipping was the most frequently reported blood sampling method and the one that the authors consider safest. Eight studies did not report the blood sampling method. One study (Tiebout III 1992) disclosed bird mortality. In spite of the many techniques reported for obtaining blood samples from hummingbirds (Table 4), a comprehensive research investigation studying the effects (e.g., on reproductive success, survivability, and migration) of obtaining blood samples from hummingbirds still needs to be performed.

Individuals planning to obtain blood samples and mark hummingbirds should apply for authorization on their BBL permit. Blood sampling should be performed only by individuals who have expertise in handling and working with hummingbirds, because the proper handling and monitoring of birds during these procedures are just as critical as the blood collection itself. In addition, a hummingbird's relatively small total blood volume means there is little margin for error in sampling.

Although blood sampling by venipuncture has been described in hummingbirds (e.g., by puncturing or sampling from the brachial/ulnar, tarsal, or femoral veins), the authors do not endorse this method due to the risk of fatal hemorrhage or secondary injury (e.g., broken wing). In addition, the superficial location of the jugular vein provides limited soft tissue support during a hemorrhagic event, which can result in fatal blood loss. Hemorrhage post-sampling is a form of blood loss and should be avoided at all costs given hummingbirds' relatively small blood volume. At the time this manuscript was written, the BBL would authorize only toenail clipping as a means of obtaining blood samples from hummingbirds and would authorize blood collection only by individuals who had received hummingbird blood sampling training from an approved trainer (B. Peterjohn, pers. comm., 28 June 2020). Blood sampling experience with other avian species is not considered sufficient to receive BBL authorization for taking blood samples from hummingbirds. Because the number of approved trainers and their time availability are limited, advance planning is imperative to receive this training.

Wearing nitrile gloves while blood sampling protects the user from hemostatic agents used to stop post-sampling hemorrhage, and gloves can be changed easily between sampling events to minimize spread of infectious diseases. A standardized guideline used by avian veterinarians and ornithologists for the amount of blood that can be sampled safely from live healthy birds is 1% of the bird's total body weight in volume during a single phlebotomy event (Lumeij 1987). However, because hummingbirds have a very small margin of safety for blood loss, this general limit does not allow for an acceptable safety factor. Considerations must include secondary post-sampling hemorrhage due to the sampling method, limited investigator experience, and the health and migratory status of the bird. Therefore, the authors recommend a more conservative sampling limit of 0.5% to 1% total lean body weight (in volume) for the individual hummingbird species. The precise amount depends on the skill level of the individual performing the sampling, the blood sampling technique, the bird's condition, and the environmental conditions that might impact the bird's physiologic and immunologic status. The lean body weight used for calculations should be reference values for the species, because some hummingbird species accumulate considerable adipose stores during migration. For example, the acceptable range in blood sample volume for a 3.5 g hummingbird is 17.5 to 35 µL. The 35 µL volume should be used only for bleeding methods after which hemorrhage is expected to be minimal (i.e., a toenail clip), if the bleeder has extensive experience, and if the bird is in very good body condition. Note that hummingbirds living at high altitudes may require special consideration. Even though it has been shown that hemoglobin has increased affinity for oxygen in hummingbirds inhabiting high altitudes (Projecto-Garcia et al. 2013), it is unknown how blood sampling impacts a live bird that is released after sampling. Therefore, a more conservative sample volume limit is advised and should be tested incrementally (e.g., starting with

Table 4. Summary of methods used to obtain hummingbird blood samples as reported in published studies.[a]

Bleeding method	Count	Volume per bird	Applications	References
Brachial venipuncture	6	1–200 µL	Endocrinology, genetics, pathology	Williams 1978; Hiebert et al. 2000b; Gregory et al. 2009; Projecto-Garcia et al. 2013; Matta et al. 2014; González-Gómez et al. 2015
Ulnar venipuncture	1	30-200 µL	Genetics	Projecto-Garcia et al. 2013
Femoral venipuncture	1	15 µL	Genetics	Roy et al. 1998†
Jugular venipuncture	1	70-100 µL	Energetics	Weathers and Stiles 1989
Tarsal venipuncture	2	30 µL	Endocrinology	González-Gómez et al. 2014a, b
Toenail clip	12	10-50 µL	Energetics, endocrinology, isotope analysis, clinical pathology, parasitology, physiology	Bakken et al. 2004; Bakken and Sabat 2006; Hardesty and Fraser 2010; Fernández et al. 2011a, b; Matta et al. 2014; González-Gómez et al. 2015; Hagadorn et al. 2016; Bradshaw et al. 2017; Safra et al. 2018; DeRogatis et al. 2020; Godwin et al. 2020
Unreported method	8	20-40 µL	Energetics, genetics, physiology	Tiebout and Nagy 1991; Tiebout III 1992‡; Chaves et al. 2007, 2011; Parra et al. 2009; Chaves and Smith 2011; Harrigan et al. 2014; Wright et al. 2014

[a] Literature searched until September 2020.

†Specifically reported that no birds died during bleeding.

‡Reported bird deaths associated with blood sampling.

0.25% of body weight followed by post-sampling monitoring) before sampling numerous birds.

In addition to the amount of blood sampled, the authors recommend considering the timing of blood sampling. Researchers should avoid times when birds are at increased risk of hypothermia or hypoglycemia (early morning, evening, cold temperatures) or when ambient temperatures limit blood distribution to the extremities. The range of temperatures in which blood sampling should be avoided also depends on other variables, such as humidity or wind. In the author's experience (L. Tell), ambient temperatures of 50° F (10° C) or lower, with no other variables, indicate that blood sampling should either be postponed, performed in a warmer environment, and/or the bird should first be slowly warmed with supplemental heat before sampling. In cool ambient temperatures, peripheral vasoconstriction may necessitate clipping a large distal portion of the toenail, thus risking amputation of the phalangeal bone. Best practice suggests forgoing sampling if blood does not flow from a conservative toenail clip. Alternatively, a supplemental heat source can be used to facilitate vasodilation in the extremities. However, the heat source must be at a relatively low setting to avoid overheating and to prevent a large temperature difference between sampling and release to the wild. Heat sources intended for young animals (e.g., puppies or kittens) or those that can be placed in an animal crate typically have low settings. If the ambient temperature is cool in the early morning but rises later in the day, it is very important discontinue use of the external heat source. Birds should never be left unattended while lying on a heat source. Birds that are overheating will open-beak breathe (pant) or will move around more than usual; however, birds showing none of these signs may still be overheating. Therefore, the bird, the ambient temperature, and the heat source temperature must be monitored continuously.

The small body size and limited total blood volume of some hummingbird species also means there are limits on repeated blood sampling, especially within a short time period. A minimum of 7–10 d from a previous bleeding event should pass before a bird is resampled. This will give the hummingbird a chance to recover and compensate for blood loss. Even though permitting agencies do not require marking a bird that has been blood sampled, it is recommended (L. Tell) that any un-banded hummingbird being blood sampled be marked in a subtle way, such as minimal clipping of a feather. The marking method does not have to be permanent (such as leg banding) for purposes of short-term identification, especially if birds in the area are not monitored on an ongoing basis.

4.2. Toenail Clipping Method for Obtaining Blood Samples from Hummingbirds

Toenail clipping is the predominant and safest method for obtaining blood samples from live hummingbirds, especially for researchers who are not experienced with blood sampling. To obtain a blood sample from the toenail, the authors recommend using a mesh bag made of seine material (see section 2.3) to help restrain the bird and allow for easy access to the toenails. The bird is restrained within the mesh bag in a supine position with both wings positioned against its body. The researcher then folds the long sides of the mesh bag underneath the bird so that the bird is confined to a limited space (Fig. 6). The bird is then placed on

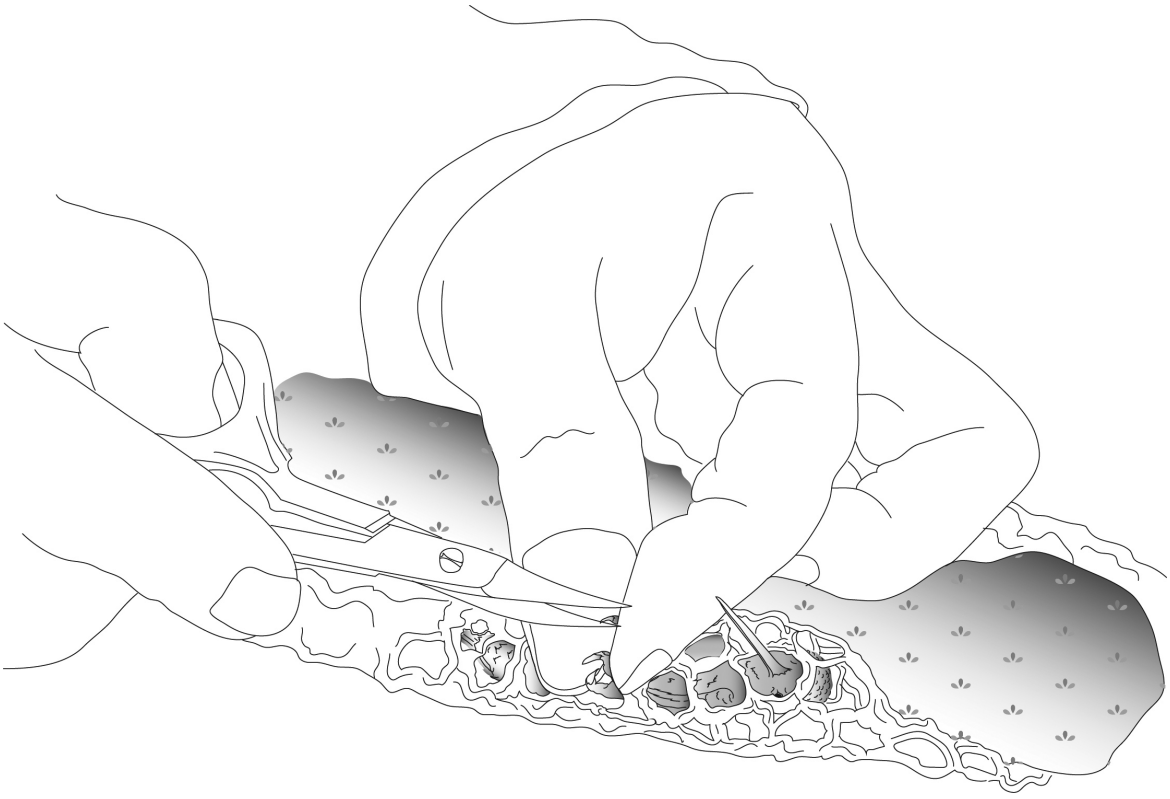

Figure 6. Illustration of the toenail clip blood-sampling method for obtaining a blood sample from a hummingbird that is restrained in a seine mesh bag. An oblong "bean bag" is used to rest and steady the researcher's left hand and also minimize bird movement within the holding bag. Curved cuticle scissors (with scissors positioned in a concave "up" position) allows clear view of the toenail to be clipped. Because the researcher's left hand is resting on the "bean bag", the thumb and second fingers that are restraining the toenail are not placing any pressure on the bird's chest.

a flat surface, and a weighted object is placed next to the bird to constrict bird movement in the bag. So as not to impair respiration, the weighted object should not be placed on top of the bird or too tightly against the bird's body. In Figure 6, the weighted object is an oblong beanbag constructed of a four-way stretchable polyester fabric stuffed with small polystyrene beads. The small beads are lightweight and conform easily to the bird's side. The weighted bag also provides a structure on which to rest a hand while obtaining the blood sample so that the hand does not lean on the bird's body.

Commercial stainless steel, curved-blade scissors (i.e., Revlon® curved-blade cuticle scissors, Revlon, Inc., New York, NY) are ideal for cutting hummingbird toenails for blood sampling because the curved blade facilitates visualization of the toenail tip. The stainless steel ensures that scissors are sharp, even after continuous use, and helps achieve a clean cut. Before sampling, scissors should be cleaned with a sanitizer (i.e., Super Sani-Cloth® germicidal disposable wipe, PDI, Woodcliff Lake, NJ), rinsed with water, and dried. Researchers can use their fingernails to gently grasp a toenail and pull the foot out through an opening in the bird bag. Caution should be used when grasping the toenail, because excessive manipulation can avulse the keratin sheath.

Once the foot is accessible, it is held at the tarsal joint. The second, third, and fourth digits can be then be spread out and controlled using the researcher's fingers—thus keeping them protected—while the toenail of the third digit is being cut. The researcher should be aware of hand placement so as not to put pressure on the bird's body and restrict respiration. Based on experience (L. Tell), the toenail of the third digit is recommended for blood sampling because it is the longest nail. The distal aspect of the toenail is cut perpendicular to the digit, removing a maximum of 5% to 10 % of the total nail length. Although it is unknown if the phalangeal bone is cut during this process, the risk of amputating the phalangeal bone increases as more toenail is cut. Amputation of the phalangeal bone is painful for the hummingbird. Using magnification during this procedure helps the researcher visualize toenails of digits two and four while the toenail of digit three is cut.

If blood does not flow after the toenail tip is cut, one should ensure that blood flow is not being impeded by finger restraint of the toe before cutting more of the toenail. Even a slight amount of finger pressure when holding a hummingbird's foot or toe can impede blood flow. With the fingers still in place around the toes, very gently release and re-apply pressure to the toes to facilitate blood flow.

As discussed above, cool ambient temperatures also can impact blood flow from a cut toenail. Cold ambient temperatures result in peripheral vasoconstriction of the extremities; therefore, attempting blood sample collection via toenail clipping at temperatures below 10° C (50° F) is not advised. At low temperatures, more of the toenail will need to be cut for blood sampling, which increases the risk of phalangeal bone amputation. In the presence of cooler ambient temperatures, a supplemental heat source can be helpful (see section 4.1). In contrast, warm ambient temperatures result in vasodilation and blood will flow freely from the clipped toenail. Therefore, it is imperative to have hemostatic materials readily available after the toenail is clipped. Until hemostasis is achieved, firm digital pressure on the foot or toes also can help restrict blood flow.

Absorbent materials used for blood storage are ideal for sample collection because they can be blotted against the cut toenail tip to facilitate blood collection; examples include Whatman® Flinders Technology Associates (FTA) sample cards (GE Healthcare, Wauwatosa, WI) or filter paper strips (i.e., Advantec™ Nobuto blood filter strips, Cole-Parmer, Vernon Hills, IL). If lysis buffer is used, it is easiest to collect the blood sample using a laboratory grade pipette (volume capacity 0–20 µl) and ejecting the blood directly into the center of the tube containing the buffer. Another option for obtaining a blood sample is to place the lumen of a capillary tube at the site of the cut toenail. This works best with warm ambient temperatures because blood flow is typically consistent. Blood smears can be made by blotting a glass slide against the site of the cut toenail or by letting a drop of blood fall onto the coverslip.

Once the sample is collected, a hemostatic agent such as silver nitrate or styptic powder is applied to the cut toenail. Even if blood did not flow from the

cut toenail or if bleeding appears to have stopped, a hemostatic agent always should be applied to prevent unexpected bleeding post-release. Styptic powder does not tend to adhere to the tip of a hummingbird's cut toenail (L. Tell). Therefore, the authors recommend direct application of silver nitrate using a cautery stick. A prescription from a veterinarian is needed for ordering silver nitrate cautery sticks. When using silver nitrate sticks, the silver nitrate end needs to be held directly and firmly against the toenail tip while the stick is rotated. The researcher's skin is protected by using a nitrile-gloved hand to hold the toenail.

To assess whether the toenail can be cut without amputating a portion of the phalangeal bone, the authors used five hummingbirds that were being euthanized for other reasons. The tip of the third toenail was cut, simulating toenail blood sampling in the field. After euthanasia, the foot was immediately fixed in 10% neutral formalin. The toes were processed through alcohol and xylene solutions and embedded in paraffin, ensuring that the digits were placed on their sides and tamped down for straightening as the paraffin cooled. Toes were cut in 4-micron serial sections for complete microscopic examination of the entire distal phalanx. Sections were stained with hematoxylin and eosin and examined. Four of five cut toenails did not have phalangeal bone imposition (Fig. 7A), whereas one toenail had evidence of bone amputation (Fig. 7B). All capture, handling, sampling, and euthanasia procedures for this work was approved by the University of California-Davis IACUC and were conducted under authorization of federal and state scientific permits (LAT; USGS BBL permit #23947, US Fish and Wildlife Scientific Collection permit MB55944B-2, and California Department of Fish and Wildlife SC-013066).

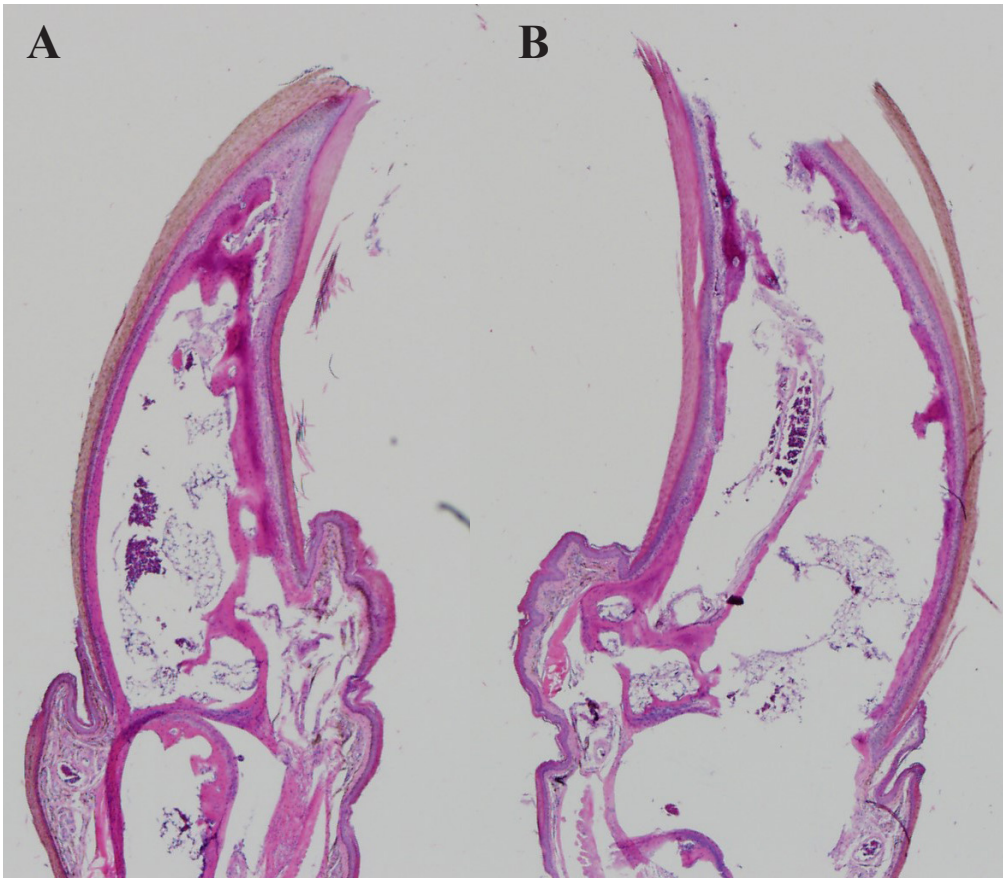

Figure 7. A) Hummingbird's (subject #1) distal phalanx of third toe after the keratin sheath of the toenail was cut. Hematoxylin and eosin stain. B) Hummingbird's (subject #2) distal phalanx of third toe demonstrating the bone of the distal phalanx was cut. Hematoxylin and eosin stain.

Advantages to the toenail clip method for obtaining blood samples are that there is a low probability of hematoma formation (swelling of blood within tissues or under the skin) and a high probability of hemostasis at the venipuncture site following cauterization. The method is relatively safe compared to blood sampling from soft tissue sites. In addition, if the bird is sampled when the ambient temperature is at least 10° C (50° F), the maximum blood volume allowed for a single bleeding event is achievable. There are three disadvantages to the method: 1) with excessive and repeated manipulation of the toenails, the keratin sheath of the toenail might be mistakenly avulsed while trying to extract the toenail from an opening in the mesh bag; 2) if the bird is cold, the blood flow rate might be less than with soft tissue venipuncture, and excessive cutting of the toenail might result in painful amputation of the phalangeal bone; and 3) there is risk of human skin exposure to silver nitrate if cautery sticks are being used without a gloved hand.

4.3. Blood Specimen Storage and Analysis

A literature review revealed 18 manuscripts that described seven methods for storing hummingbird blood for later use (Table 5). The sample storage method is highly dependent upon the research application. For genetic analysis, some investigators prefer using Whatman® FTA sample cards (GE Healthcare) due to the ease collecting blood onto a card when using a toenail clip bleeding method and the stability of the sample at room temperature. For general blood storage or testing for infectious diseases, blood filter strips work well due to ease of blotting blood onto the strip when using the toenail clipping blood collection technique and the ability to distance blood spots along the strips.

For analysis of blood smears for estimated white blood cell counts and the presence of hemoparasites, the cover slip method for making blood smears is useful when only a small volume of blood has been obtained during a toenail clip. This technique puts minimal traumatic force on the cells if the individual making the slide has limited experience. The authors found that using two medium-sized (24 mm x 50 mm) versus small-sized (20 mm x 20mm) cover slips facilitates making a feathered monolayer blood smear because there is additional space for holding the cover slips as they are separated. Helpful field guidelines for making and staining blood smears for species with nucleated red blood cells can be found at https://www.uvm.edu/~jschall/techniques.html. A modified Wright's stain (Camco Quik Stain®, Cambridge Diagnostic Products, Inc., Fort Lauderdale, FL) is a commercially available rapid stain that works well with hummingbird blood smears (Safra et al. 2018). Figure 8 details a protocol for staining hummingbird blood slides that is modified from the manufacturer's recommendations.

5. Obtaining Feather Samples from Hummingbirds

5.1. Trends and Applications

A review of the literature regarding feather sampling from hummingbirds revealed 33 results and revealed trends in feather type, quantity, and application used (Table 6). More research is needed to compile best practices for feather sampling from hummingbirds. As with all avian species, hummingbird feathers have critical behavioral, thermoregulatory, and physiologic functions (McDonald and Griffith 2011). Thermoregulation especially is important for hummingbird survival and should not be compromised. Studies evaluating the impacts of molt on bird health and survival in other bird species have shown significant costs in flight maneuverability after feather loss and energy expenditure during feather regrowth (Swaddle and Witter 1997; Bridge 2004). One investigator documented that removing all of a hummingbird's tail feathers slightly reduced its flight maneuverability (Clark 2011a). Feathers also serve important communication functions; for example, males of many hummingbird species can produce courtship sounds through aeroelastic tail flutter during the breeding season (Clark et al. 2018a; Clark and Mistick 2018b). Therefore, it is very important to be thorough in evaluating which hummingbird feathers and how many can be removed for sampling.

Table 5. Summary of methods used to store hummingbird blood or urine samples as reported in published studies.[a]

Sample type	Count	Storage method	Temperature	Applications	References
Blood	1	Nobuto blood strips	Not reported	Genetics	Hagadorn et al. 2016
Blood	3	Whatman FTA Elute cards	Not reported	Phenology	Correa-Lima et al. 2019; Baek et al. 2020; Godwin et al. 2020
Blood	5	Sealed capillary or microhematocrit tubes	-80° C to 5° C	Endocrinology, energetics	Weathers and Stiles 1989; Tiebout III 1992; Fernández et al. 2011a, b; González-Gómez et al. 2014a
Blood	3	Centrifuged immediately and plasma extracted then frozen	-80° C	Energetics, physiology, genetics	Hiebert et al. 2000c; Bakken and Sabat 2006; Projecto-Garcia et al. 2013
	1	1.5-mL tube in 99% ethanol	Not reported	Isotope analysis	Hardesty and Fraser 2010
Blood	1	SET buffer	-20° C to ambient room temperature	Pathology	Matta et al. 2014
Blood	5	Blood smear	-20° C to ambient room temperature	Genetics, pathology	Williams 1978; Gregory et al. 2009; Matta et al. 2014; Wright et al. 2014; González-Gómez et al. 2015
Urine	1	Frozen in 1.8-mL cryotube with tris borate EDTA buffer	20° C	Pathology	Henning et al. 2015
Urine	1	Cryotube frozen in liquid nitrogen	-80° C	Pathology	Williams et al. 2012
Urine	2	Microcentrifuge tube, centrifuged, then frozen	-20° C	Endocrinology	Hiebert et al. 2000a, b
Urine	2	Nalgene or other tube frozen	-20° C	Physiology	López-Calleja and Bozinovic 2003; McWhorter et al. 2003
Urine	1	Dried for weight	60° C for 6 days	Energetics	López-Calleja and Bozinovic 2003
Urine	1	Frozen in 1-mL vials	-5° C	Toxicology	Bishop et al. 2018
Urine	2	Flame-sealed capillary tube	Not reported	Energetics	Barbachano-Guerrero et al. 2019; Shankar et al. 2019

[a] Literature searched until September 2020.

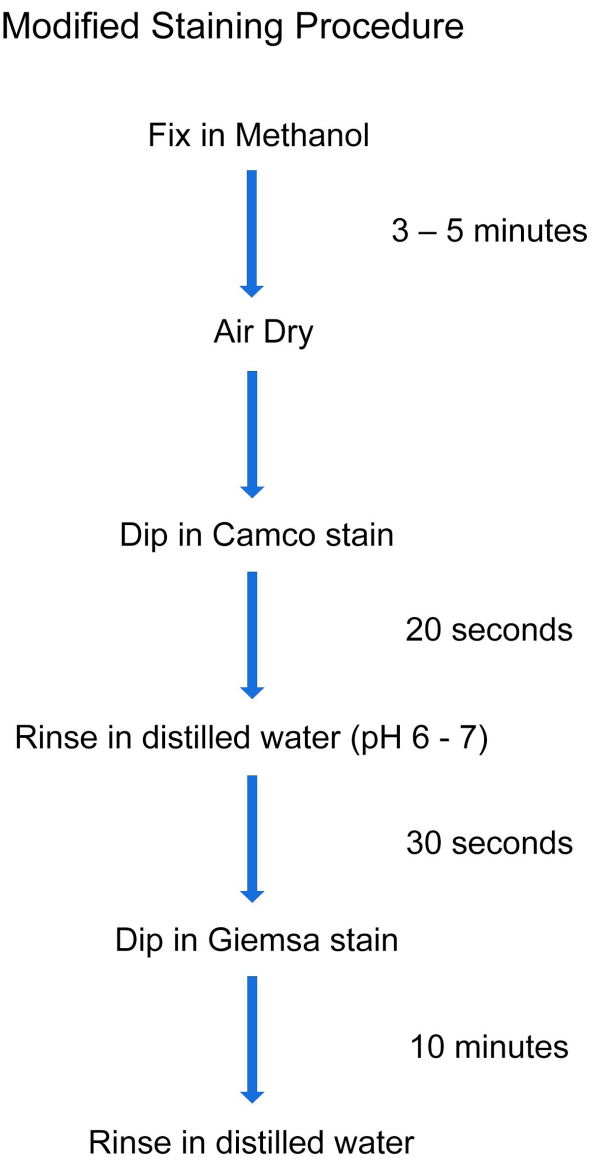

Figure 8. Suggested protocol for using a commercially available modified Wright's stain to stain cells on blood smears from hummingbirds.

Table 6. Summary of methods used to collect hummingbird feather samples as reported in published studies.[a]

Feather source		Count	Quantity	Applications	References
Tail	R4	2	1 feather	Isotope analysis	Brown et al. 2012; Moran et al. 2013
	R5	3	2 feathers	Genetics	González and Francisco Ornelas 2014; Licona-Vera and Francisco Ornelas 2014; Rodriguez-Gomez and Francisco Ornelas 2014
	R4 and R5	4	2 feathers	Physiology	Clark 2011b; Clark et al. 2011a, b, 2012
	R3 and R5	1	1 feather	Genetics	Malpica and Ornelas 2014
	R1	2	1 feather	Color spectrometry, viral DNA detection	Meadows et al. 2012; Baek et al. 2020
	Varied	2	1–2 feathers	Trace element analysis, Tabletop scanning electron microscopy	Mikoni et al. 2017; Yamasaki et al. 2018
	Unspecified	6	2 feathers	Genetics, isotope analysis	Hardesty and Fraser 2010; Gonzalez et al. 2011; Rodríguez-Gómez et al. 2013; Ornelas et al. 2016; Hernández-Soto et al. 2018; Rodríguez-Gómez and Ornelas 2018
Head/crown		5	5 feathers	Color spectrometry	Parra et al. 2010; Meadows et al. 2011, 2012; Dongen et al. 2013; Eliason et al. 2020
Back		3	5 feathers	Color spectrometry, genetics	Parra et al. 2010; Dongen et al. 2013; Eliason et al. 2020
Chest		4	5 feathers	Isotope analysis, genetics, toxicology	Brown et al. 2012; Dongen et al. 2013; Hagadorn et al. 2016; Sierra-Marquez et al. 2018
Chest		2	Multiple feathers	Trace element analysis, viral DNA detection	Mikoni et al. 2017; Baek et al. 2020
Gorget		4	5 feathers	Color spectrometry	Parra 2010; Meadows et al. 2011, 2012; Eliason et al. 2020
Back		2	5 feathers	Color spectrometry, genetics	Parra 2010; Dongen et al. 2013
Primaries and secondaries		1	2 feathers	Mite study 96% ethanol	Mironov et al. 2019
Unspecified feathers		7	2 feathers	Genetics, irredescence	Roy et al. 1998; Hardesty and Fraser 2010; González-Gómez et al. 2011, 2013; Gonzalez et al. 2011b; Ornelas et al. 2016; Sosa et al. 2020

[a] Literature search until September 2020.

5.2. Methods for Sampling Feathers from Hummingbirds

5.2.1. Appropriate timing for feather sampling: molt considerations.—Migratory North American hummingbirds exhibit a molting strategy that is similar across species. Most of these species undergo a pre-basic molt on their wintering grounds beginning in August to October and ending by January to March (Pyle 1997). Within these dates, there is some variation among species. For example, some southwestern species (e.g., *Calothorax, Eugenes, Cynanthus*, and *Lampornis*) can have protracted molts that extend into April or May. Exceptions to this molting strategy are the *Calypte* and *Amazilla* species in which pre-basic molt is primarily from June to September (Pyle 1997; Pyle et al. 1997). Anna's Hummingbirds (*Calypte anna*) can have a protracted molt lasting from May through January (Williamson 1956). Flight feather replacement is sequential in all species, and sequential replacement of tail feathers begins sometime after primaries are replaced. A recently discovered hatch year, second pre-basic molt in winter has been found in Ruby-throated (*Archilochus colubris*) and Rufous Hummingbirds (*Selasphorus rufus*), but this should not alter the timing of feather sampling (Dittmann and Cardiff 2009; Sieburth and Pyle 2018).

5.2.2. Appropriate timing for feather sampling: other considerations.—Considerations regarding the timing of feather sampling should minimize impacts on the reproductive activities (e.g., courtship) and thermoregulation of hummingbirds. Thus, sampling should be synchronized to the expected breeding and molting periods of their life cycle. For adult male and female North American hummingbird species, feather sampling is best limited to the period between the end of nesting and the start of molting, which is between July and September for most species. For example, removal of an adult male's iridescent head or gorget feathers should be avoided during the breeding season. In addition, tail feathers and flight feathers from adult males can be sampled after the breeding season and before the winter molt without affecting courtship behavior. Species-specific nesting periods can be ascertained by using breeding season data in the *Birds of the World* series (Cornell Lab of Ornithology) and molting dates can be obtained (Pyle 1997; Pyle et al. 1997). Flight feathers should not be sampled during migration, but sampling can be considered during winter when birds are molting. Sampling of primary feathers should be limited to P1 and P2 (Fig. 5) unless birds are in primary molt when older outer primaries can be sampled. When considering sampling tail feathers, R2 and R3 (Fig. 5) are recommended at all times.

Special consideration should be given to sampling hatch year (HY) birds. Because these birds require intact flight feathers prior to migration, they have especially critical energy demands. In some species, HY birds that arrive in wintering grounds undergo a second pre-basic molt and replace all of their flight feathers (Sieburth and Pyle 2018), thus following the same molting schedule as adult birds. Sampling old feathers at this time is acceptable. After molting in the wintering grounds, HY birds attain adult plumage and should not be sampled until after the breeding season. The researcher should be familiar with the dates of migration, courtship, and nesting of the hummingbirds involved in their study in order to select the least invasive time for feather sampling. In addition to assessing the risks associated with removing a certain type of feather from a given species, knowledge of the proper technique for feather sampling is paramount. Hummingbird skin is delicate. If feathers are pulled too aggressively or in the wrong direction, the skin can tear, necessitating closure with suture.

5.2.3. Methods for sampling contour feathers.—When sampling contour feathers, the recommendation is to collect a total of 20 to 30 feathers from four different quadrants and feather tracts in the chest region. Feathers should be removed without creating patches of exposed skin that could compromise thermoregulation. This is especially important during winter months or at high elevations when/where birds encounter low temperatures. Ideally, feathers are removed at scattered locations over the bird's body so that normal preening can cover the sampled areas. However, research is still needed to evaluate the impacts of feather sampling on hummingbirds. A hemostat or blunt-tipped forceps is recommended for obtaining feathers. Both are efficient for sampling contour feathers and minimize the chance of inadvertently grasping skin during the sampling process. During feather sampling, the bird is held in a supine position in a cupped hand (Fig. 9). The bird's

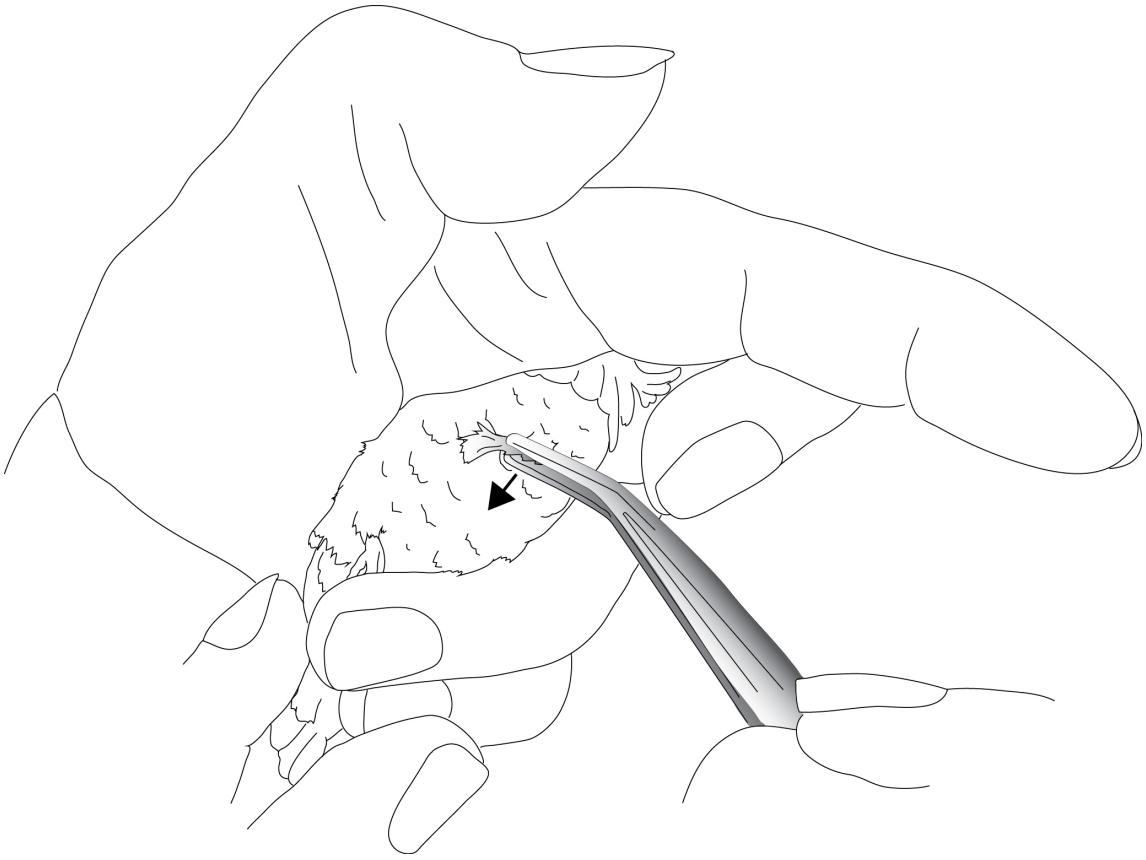

Figure 9. Illustration of sampling covert feathers from the pectoral region of a hummingbird. Note that the bird is being restrained using the hummingbird bander's hold with the fourth and fifth fingers restraining the feet. The forceps are held in a horizontal position relative to the long axis of the bird's body, which helps minimize the chances for inadvertently pinching the skin. When sampling, the feathers are grasped within the opening of the forceps. The forceps are moved in a downward direction (in the direction of the arrow) to minimize the chance of inadvertently tearing the skin when feather sampling. The feathers should be placed in an acid free envelope to maximize the duration of sample usefulness for scientific studies.

feet are secured so that they are not inadvertently harmed during the sampling process. The instrument used to pull feathers (e.g., forceps or a hemostat) is held at the proximal end of the feather shaft (i.e., close to where the feather attaches to the skin) with the instrument tip positioned perpendicular to the long axis of the bird (Fig. 9). Pulling feathers down and away from feather follicles minimizes the risk of inadvertently lacerating the skin. Before sampling, it is important to visually confirm that no skin is contained within the forceps or hemostat.

5.2.4. Methods for sampling wing and tail feathers.—In general, protocols for sampling hummingbird wing and tail feathers should be similar to guidelines provided for sampling these feathers in passerines (Fair and Jones 2010). An important exception involves the possible sampling of tail feathers that are important to hummingbird males during the breeding season. Researchers also must consider the metabolic expense of feather replacement for hummingbirds, a species that already has significant energy demands.

Removal of a wing or tail feather can be accomplished without using an instrument. Secure the feather base between the thumb and forefinger and pull gently but firmly while using the fingers from another hand to stabilize the anatomic region where the feather is attached to the body. For the safety of the bird and to ensure proper removal, grasp the feather at the proximal end (base) of the feather shaft during removal. If a wing feather is sampled, the wing is stabilized with the fingers of the opposite hand so that the bones are not accidentally fractured during the feather removal process. As mentioned previously, knowledge of the acoustic function that individual feathers play in courtship and behavior is important in determining which feathers to sample (Clark 2011b; Clark et al. 2018a; Clark and Mistick 2018b). A best practice is to avoid sampling wing feathers if an alternative feather type (such as a tail feather) would suffice; that is because wing feathers are very important for flight and sometimes produce sound (e.g., in *Archilochus* spp.). Of the ten primary feathers, P10 (Fig. 5) contributes the most to production of aerodynamic force. No research studies have been conducted to determine if tail feather sampling affects the overall reproductive fitness of a hummingbird population; however, sampling tail feathers other than the acoustic tail feathers is best practice. If females preferentially select a male based on the sounds made by tail feathers, the change in fitness for one male will most likely result in a fitness increase for another male. Therefore, while there might not be a net change in fitness for the population as a result of sampling tail feathers, fitness for the individual sampled bird might be altered due to human intervention.

5.2.5. Methods for sampling blood feathers.—Compared to mature feathers, blood feathers—which contain pulp tissue and blood—offer a more reliable source of deoxyribonucleic acid (DNA). Sampling blood feathers requires blood sample authorization either on BBL or USFWS Migratory Bird Scientific Collecting permits, depending on whether the bird is to be banded or not. Because blood feathers have an active blood supply, they must be removed in their entirety. Removal of a blood feather should be performed only by an individual with experience in removing blood feathers. When a blood feather is removed, the feather should be examined carefully to ensure that the entire base of the feather shaft was removed; this will ensure involution of the skin follicle's vascular supply. If the entire shaft of the blood feather is not removed, fatal hemorrhage can occur. If any part of the shaft remains and has sufficient integrity, micro-hemostats can be used to grasp and remove the remaining tissue. Magnification is helpful during this process. Once the entire blood feather has been removed, digital pressure can be applied to the feather follicle. To avoid wing bone fractures, special care must be taken when sampling wing feathers. Digital pressure should be released if the bird attempts to flap its wing; pressure can be reapplied once the bird is calm. Because blood feather removal can cause temporary discomfort and has inherent risk, the researcher's skill level and necessity/value of the blood feather sample should be considered before sampling.

The total blood volume in a blood feather from a hummingbird varies depending on the maturity and size of the feather. Because early-developing wing and tail blood feathers potentially have the greatest blood volume, it is advisable for a researcher to sample only one wing feather or tail feather from a hummingbird during a single encounter. Further recommendation is to wait at least 7–10 d before sampling another large blood feather from that individual.

5.3. Methods for Feather Specimen Storage and Analysis

Most studies reviewed did not specify how feather samples were stored; however, those that did used paper receptacles, such as coin envelopes. Acid-free envelopes are recommended, and silica gel packs help minimize moisture. A sample identification number can be assigned to the envelope, which is cross-referenced to the sampled bird by using a unique catalog number. It is best to label envelopes in pencil and then place tape over the writing to avoid smearing. Alternatively, feathers can be stored taped to 3-in x 5-in archival cardstock, which is sufficient to hold the entire set of tail feathers from a single hummingbird. The card is then slipped inside a 3-in x 5-in archival glassine envelope, which will help minimize specimen damage. These samples can then be stored inside a 3-in x 5-in card box. Foil or plastic bags also can be used to store feathers. Selection of the storage system to use will depend on the intended study (Espino-Espino et al. 2014). Although it is possible to extract genetic material from feathers

maintained at room temperature, there is anecdotal evidence (L. Tell) that freezing hummingbird feathers at -20° C or -80° C may help minimize DNA degradation over time. If envelopes will be frozen or exposed to moisture or humidity, the glue on the envelope flap may not hold. Applying a small piece of adhesive tape to the envelope seal will help prevent the loss or mixing of samples.

6. Obtaining Oral or Cloacal Swabs from Hummingbirds

6.1. Trends and Applications

Review of the literature found two published studies mentioning oral and cloacal swab sampling on hummingbirds (Williams et al. 2012; Barbachano-Guerrero et al. 2019); however, the materials and methods were not specified. Oral cavity and cloacal swabbing are commonly performed in other avian species to obtain samples for determining normal oral flora or for pathogen (bacterial, yeast/fungal, parasitic, or viral) testing. Swabbing the mouth or cloaca is not performed routinely with hummingbirds due to the fragility of the beak/tongue and the lack of a small enough swab tip for insertion into the hummingbird cloaca.

6.2. Methods for Oral and Cloacal Swabbing

Given the previously mentioned challenges, methods for obtaining oral or cloacal swabs from hummingbirds are not well-established. For obtaining samples from the proximal gastrointestinal tract, palpating the crop to induce fluid regurgitation is not recommended, because the bird could aspirate a small amount of fluid (that is not visually obvious) and the health ramifications would not manifest until 24–48 h after the bird is released. An alternative to oral cavity swabbing is to have a hummingbird drink from a small vial filled with sterilized sugar water and then sample from a small volume of sugar water that was not ingested (Lee et al. 2019). Depending on the study goals, this method might provide samples representing the oral microbiota. An alternative for cloacal swabbing might be to use the tip of a cellulose eye spear (i.e., Weck-Cel®cellulose eye spears, BVI Medical, Waltham, MA). These sponges are made from highly absorbent natural cellulose material and are designed for use in delicate surgical areas.

7. Obtaining Cloacal Excreta Samples from Hummingbirds

7.1. Trends and Applications

A literature review yielded 21 results for cloacal excreta sampling in hummingbirds as well as trends in sampling techniques and applications (Table 7). Collecting excreta (urine and/or feces passing through or having exited the cloaca) from a confined but unrestrained bird is a non-invasive method for urine and/or fecal sampling. Urine or feces also can be collected from a restrained bird.

When measuring analytes in urine samples from birds, there are some fundamental differences between urine samples obtained directly from the ureters versus the cloaca. Solute concentrations in cloacal urine can differ substantially from ureteral fluids released by the kidney despite birds not having a bladder (that results in urine pooling) nor substantial urine concentrating capabilities (Lotz and Martínez Del Rio 2004) compared to mammals. The reason for the cloacal urine to differ from the ureteral urine is attributed to dietary water excreted from the gastrointestinal tract into the cloaca, because avian gastrointestinal and urinary tracts join at the cloaca. The fluid volume that hummingbirds drink and excrete through their gastrointestinal tract could have a substantial impact on cloacal urine solute concentrations, as has been shown in sunbirds (McWhorter et al. 2004). For mammalian urine samples, creatinine typically is used as an endogenous marker to normalize analyte concentrations and account for urine dilution or concentration. However, birds have limited metabolism of creatine to creatinine (Paton 1910), and therefore creatinine is not useful for normalizing urine samples. Creatine has been measured in bird urine samples by modifying a creatinine analytical method (Wimsatt et al. 2009); however, little is known about the use of

Table 7. Summary of methods used to collect hummingbird cloacal excreta samples as reported in published studies. [a]

Method	Count	Volume per bird	Applications	References
Uretal urine collected with a close-ended cannula	2	Not reported	Physiology	Bakken et al. 2004; Bakken and Sabat 2006
Microcapillary tube held up to cloaca	2	30–50 µL	Endocrinology	González-Gómez et al. 2014a, b
Micropipettes or microcapillary tubes used to collect from surface of a cage liner	10	50–1200 µL	Energetics, endocrinology, physiology	Preest and Beuchat 1997; Hiebert et al. 2000a, c; McWhorter and Del Rio 2000; López-Calleja and Bozinovic 2003; López-Calleja et al. 2003; Lotz and Martínez Del Rio 2004; Bakken and Sabat 2006; Goloff and Burch 2012; Chavez-Zichinelli et al. 2014
Cloacal fluid collected directly from the cloacal opening using 100- µL micro-pipette	2	Anna's Hummingbird (30–60 µL) Rufous Hummingbird (5–30 µL)	Toxicology for pesticides	Bishop et al. 2018, 2020
Cloacal swab	1	Not reported	Pathology	Williams et al. 2012
Fecal smears	1	Not reported	Pathology	Snowden et al. 2001
Oral and cloacal swabs (frozen in viral transport media supplemented with antibiotics (stored at -80° C)	1	Not reported	Prevalence of West Nile virus	Barbachano-Guerrero et al. 2019[†]
Oral (sugar wáter in vials)	1	1 ml offered for birds to drink. Approximately 500-900 µL of remaining sugar water in vial used for sampling.	Microbiome	Lee et al. 2019
Cloacal pellets collected from area around nest (stored at -20° C)	1	Not reported	Diet analysis	Moran et al. 2019

[a] Literature search up to September 2020.

[†]Samples were collected from birds' beaks by allowing birds to drink from 1.5-ml tubes.

creatine to normalize solute concentrations in avian urine samples. Although specific gravity, as measured by a handheld refractometer, has been used as a proxy for normalizing solute concentrations in urine samples from hummingbirds, George (2001) suggested that this method should not be broadly applied to urine samples from all animals. Multiple constituents in urine samples can falsely elevate or decrease specific gravity values; therefore, a validation study is necessary before using specific gravity to normalize solutes in hummingbird urine samples.

7.2. Detailed Methods for Cloacal Excreta Sampling from Hummingbirds

7.2.1. Cage liner method for excreta sampling.—Using this method, a captured hummingbird is placed in a small enclosure with a perch, a feeder, and enough space for the bird to comfortably feed. A soft mesh butterfly cage works well for this purpose. The bottom of the enclosure is lined with a material that will facilitate urine or fecal collection, such as plastic wrap or plastic-coated paper. If urine collection is a goal, micropipettes or microcapillary tubes can be used to lift excreta samples from the floor liner. Filter paper can be used to collect feces for DNA sampling, because it can be easily frozen, cut, and immersed in fluids for DNA extraction. This reduces the need to collect excreta off the paper. The cage liner method for collecting cloacal excreta from hummingbirds is easy, does not require specialized equipment, and involves very little risk to the bird. However, this method is relatively inefficient because some sample volume may be lost during transfer from paper to pipette or tube.

7.2.2. Manual restraint and capillary tube method for sampling cloacal excreta.—The method of collecting urine samples with capillary tubes, as first established in rats (Hayashi and Sakaguchi 1975), has been applied to hummingbirds (Hiebert et al. 2000a; González-Gómez et al. 2014a, b) for free-catch collection of naturally voided urine or feces. Only individuals with training and experience with handling hummingbirds should use this approach. The hummingbird is manually restrained and held in an upright position while the opening of a capillary tube is placed near the cloacal sphincter so that the voided urine or feces can be directly sampled as excreta exits the cloaca (Fig. 10). This handling technique for urine collection works for smaller-sized hummingbirds (less than 5–6 g). The hummingbird should be restrained immediately after capture so it does not void urine/feces before sample collection can occur. Fecal sample collection may be more successful later in the day rather than first thing in the morning because birds will have had more time to forage. While birds are being restrained, they should be monitored continuously and offered sugar water as needed. If urine/feces are not voided after 10–15 min of restraint, the bird should be released. Advantages to this method are that it is non-invasive, it minimizes the time required for sampling, and it eliminates the need to keep the bird in an enclosure. It is important to note, however, that even if fecal material is not present in the urine sample, the urine collected is not sterile because it is not sampled directly from the lumen of the ureters. A relatively small mass of fecal material also can be collected using this method. When using this method, the handler must be able to restrain the bird without impairing its respiration while simultaneously monitoring the bird's status and collecting samples.

7.2.3. Dish collection for excreta sampling.—A simple method for urine/fecal collection is to place hummingbirds in a mesh bag and lay them in a sitting position on a plastic, large-mesh grating over a dish. Typically, hummingbirds will produce an ample urine sample within 15–20 min, which can be collected in a capillary tube from the bottom of the dish. Urine collection is most effective when trapping periods occur in the morning after the intense period of post-roost feeding when hummingbirds are well hydrated.

7.3. Cloacal Excreta Specimen Storage and Analysis

Most of the reviewed papers suggest storing cloacal excreta samples in small cryotubes (1.5–1.8 mL) with or without a preservative buffer (e.g., 0.9% saline). This is followed by either urine/fecal sample separation by centrifuge or storage in a freezer at -20° C or -80° C (Table 5).

Figure 10. Illustration of urine/fecal collection from a hummingbird using a glass capillary tube that is held in close proximity to the cloacal opening. The bird is gently restrained in a cupped hand and the tail is reflected cranially and dorsally. Using this restraint method, the person sampling must constantly monitor for eye closure and overall bird status since the head is not as easily visualized when the bird is being restrained in this manner.

8. Measuring Metabolic Rates in Hummingbirds

8.1. Trends and Applications

A literature search yielded 68 published studies that used methods described in this section (Table 8). Measuring metabolic rates in hummingbirds is of broad interest to the scientific community for several reasons: 1) hummingbirds are among the smallest endotherms; 2) they have among the highest metabolic rates recorded in vertebrates (Lasiewski 1963a); 3) they are capable of using deep torpor at night (Hainsworth and Wolf 1976; Hiebert 1992; Powers et al. 2003); and 4) they are the only birds that employ sustained hovering flight (Warrick et al. 2005). Methods for measuring metabolic rates in hummingbirds are similar to those used for other animals. The biggest challenges result from the birds' small size and lack of long-term endogenous energy stores.

Table 8. Summary of methods used to measure hummingbird metabolic/evaporation rates as reported in published studies.[a] Note that some studies listed were conducted in a laboratory setting, but the methods can be applied to the field.

Measurement	Count	Protocol	Applications	References
Basal metabolic rate	12	Open-flow, positive pressure	Nighttime energy costs (laboratory)	Lasiewski 1963a, b, c; Weymouth et al. 1964; Lasiewski and Lasiewski 1967; Lasiewski et al. 1967; Carpenter 1974; Opazo et al. 2005;F ernández et al. 2011a, b; Fernandez and Suarez 2011; Shankar et al. 2020a
Resting metabolic rate	2	Open-flow, positive pressure	BMR conditions during daytime (laboratory)	Prinzinger et al. 1992; Bakken and Sabat 2007
Oxygen consumption and carbon dioxide production (including respiratory exchange rate)	18	Open-flow, positive pressure	Non-BMR conditions (field and laboratory)	Lasiewski 1963a, b; Lasiewski and Lasiewski 1967; Lasiewski et al. 1967; Wolf and Hainsworth 1972; Withers 1977a, b; Schuchmann 1979a, b; Schuchmann et al. 1979; Schuchmann and Schmidtmarloh 1979a, b; Powers 1991, 1992; Prinzinger et al. 1992; Chaui-Berlinck et al. 2002; Powers et al. 2010; Dick et al. 2020
Oxygen consumption and carbon dioxide production (including respiratory exchange rate)	1	Open-flow, negative pressure	Non-BMR conditions (laboratory)	ChauiBerlinck and Bicudo 1995
Body temperature measurement	1			Wolf et al. 2020
Nighttime normothermic metabolic rate	17	Open-flow, positive pressure	Temperature response, torpor patterns, energy storage threshold, ventilation pattern (field and laboratory)	Bartholomew et al. 1957; Lasiewski 1963b; Lasiewski and Lasiewski 1967; Lasiewski et al. 1967; Hainsworth and Wolf 1970; Wolf and Hainsworth 1972; Wolf et al. 1972; Hainsworth and Wolf 1978; Krüger et al. 1982; Hiebert 1990, 1992;Hiebert 1993a;b; Bucher and Chappell 1997;Powers et al. 2003;Eberts et al. 2019;Shankar et al. 2020a
Total evaporation rate	2	Open-flow, positive pressure	Water balance, humidity effects (laboratory)	Powers 1992;Bakken and Sabat 2007

Table 8. (cont.)

Measurement	Count	Protocol	Applications	References
Hovering metabolic rate	24	Open-flow, negative pressure, mask	Allometry, energy cost, energy metabolism, metabolic fuel use, thermogenesis (field and laboratory)	Berger and Hart 1972; Berger 1974a, b; Epting 1980; Bartholomew and Lighton 1986; Suarez et al. 1991; Wells 1993a;b; Chai and Dudley 1996; Chai et al. 1996; Chai et al. 1998; Altshuler et al. 2001; Welch Jr et al. 2006, 2007; Welch Jr and Suarez 2007; Evangelista et al. 2010; Fernández et al. 2011a, b; Fernandez and Suarez 2011; Welch Jr 2011; Chen and Welch Jr 2014; Kim et al. 2014; Groom et al. 2018; Shankar et al. 2020a
Oxygen consumption and carbon dioxide production	2	Open-flow, positive pressure, metabolic chamber	Energy cost (laboratory)	Lasiewski 1963b; Schuchmann and Schmidtmarloh 1979a
Respiratory evaporation rate	2	Open-flow, negative pressure, mask	Water loss, heat dissipation (field and laboratory)	Berger and Hart 1972; Powers et al. 2012
Field metabolic rate	5	Doubly labeled water	Daily energy expenditure (laboratory)	Powers and Nagy 1988; Weathers and Stiles 1989; Powers and Conley 1994; Shankar et al. 2019, 2020a
Daily energy intake	1	Measuring food intake	Daily energy expenditure, (laboratory)	Tiebout 1991; Tiebout III 1992

[a] Literature searched until September 2020.

8.2. Overview of Open-flow Respirometry Methods

Standard open-flow respirometry can be used to measure metabolic rates in captive hummingbirds in field settings (Lighton 2008). Open-flow respirometry can be used to measure oxygen consumption and carbon dioxide production (both measures of metabolic rate) as well as evaporative water loss. Specific open-flow configurations that are appropriate for a variety of metabolic rate measurements have been described (Lighton 2008).

Open-flow respirometry systems can employ either a positive pressure or negative pressure configuration. In a positive pressure configuration, air is pushed by a pump or compressed airline through a sealed metabolism chamber containing the hummingbird. A second airline that bypasses the metabolism chamber is required for baseline measurements of airstream gas composition. A single pump or compressed airline can be divided to provide airflow for baseline measurement and the metabolism chamber, but the flowrate must be measured separately for each line. Baseline and outgoing chamber airflow then empties into a syringe barrel where the flow can be sampled by the metabolism system (see below).

Accurately measuring flowrate is critical because errors can lead to large errors in measurement of metabolic rate. Using standard open-flow protocols, water and carbon dioxide are removed from air flowing into metabolism chambers to keep gas components constant. This can be accomplished by using absorbents (Drierite®, W. A. Hammond DRIERITE Co. LTD, Xenia, OH) to extract water and soda lime to extract carbon dioxide. Some studies omit the removal of carbon dioxide because its impact on flowrate is extremely low. Alternatively, if both the water and carbon dioxide content of the airstream are known, the flowrate can be corrected mathematically. To use this method, airstream samples must be run through both a carbon dioxide analyzer and a humidity sensor to measure the proportional contribution of water and carbon dioxide. Subsequently, the flowrate can be corrected by subtraction, using published equations (Lighton 2008). This is a useful approach when working in the field because it eliminates the need to transport and store absorbents. Because the partial pressures of gases in the airstream change with barometric pressure, flowrate is corrected to standard temperature and pressure (STP). This correction is done automatically if the flowrate is measured with a mass flowmeter. Flowrate also can be measured using a calibrated rotameter, but the STP correction will need to be done manually.

In a negative pressure configuration, air is pulled through a metabolism chamber or mask. One advantage of this configuration is that a completely sealed chamber is not required as long as the flowrate is sufficient to collect all exhaled air from the hummingbird for measurement of respiratory gas exchange (for metabolic rate) and respiratory evaporative water loss. A compressed airline cannot be used for a negative pressure system. In addition, pumps must be completely sealed because the metabolism system must sample air from the outlet side of the pump. Further, separate pumps must be used for baseline sampling and each chamber/mask. All requirements for accurate measurement of flowrate described above for a positive pressure design also apply to a negative pressure configuration.

Regardless of whether a positive or negative pressure design is used, baseline and chamber outlet air is sampled from the syringe barrel at the end of the open-flow system. Air is pulled from the syringe barrel by a sub-sampling pump. The flowrate of the sub-sampling pump must be less than the flowrate into the syringe barrel to prevent dilution of the sample with ambient air. The sub-sampled air can then be pushed through analyzers for measurement of the gas composition. If water and carbon dioxide are being subtracted to correct the flowrate of the main pump, the system also must include both a humidity sensor and a carbon dioxide analyzer in addition to an oxygen analyzer.

Pumps and mass flowmeters/controllers can be purchased from a variety of sources. When purchasing a pump, consider the maximum flow needs as well as the altitude at which measurements will be made. In most pumps, airflow is generated by a diaphragm and the maximum flow will be reduced by lower air density at higher altitudes. The analyzer manufacturer most commonly used for open-flow respirometry is Sable Systems International (North Las Vegas, NV), which sells analyzers in a variety of configurations, includ-

ing all-in-one systems that are easily transportable and can run for extended periods on batteries. The Sable Systems field equipment is also user-serviceable such that minor repairs can be done in the field without voiding the warranty.

Depending on the computer systems being used, there are several options for data acquisition and analysis software for open-flow respirometry. Commercial software (Expedata Data Analysis Software, Sable Systems International) integrates well with computers that use Microsoft Windows as their operating system. Warthog software, written by Dr. Mark Chappell at the University of California, Riverside, and available at no charge at warthog.ucr.edu, is well suited for Macintosh-based systems.

8.3. Measuring Basal and Resting Metabolism

Measurements of basal and resting metabolic rates in captive hummingbirds can be made using the open-flow respirometry methods described above. Few studies have measured the true basal metabolic rate (BMR)—i.e., the metabolic rate during the rest phase (nighttime for hummingbirds), in the dark, within the thermoneutral zone, and during the post-absorptive phase (i.e., after digesting food) (Carpenter and MacMillen 1976; López-Calleja and Bozinovic 1995; Opazo et al. 2005; Fernández et al. 2011b). This is likely due to the inherent difficulties associated with measurement of BMR (McKechnie and Wolf 2004), which are further amplified by the small size of hummingbirds.

Unlike BMR, resting metabolic rate (RMR) is referenced frequently in the literature. However, RMR has many different meanings because it has been measured over a wide range of temperatures, during the birds' active phase, and during periods of food digestion. Thus, even though RMR measurements in hummingbirds have been reported in a number of studies (Lasiewski 1963b; Lasiewski et al. 1967; Hainsworth and Wolf 1970; Krüger et al. 1982; Powers 1991; Powers et al. 2003), there is no standard methodology or terminology for measuring RMR. Therefore, when measuring and reporting RMR, it is important to clearly define what is being measured. Confusion sometimes can be avoided by using terminology other than "RMR". For example, when measuring RMR in Anna's Hummingbirds (*Calypte anna*), Pearson (1950) simply referred to evaluating metabolic rates while birds were "asleep" (Pearson 1950).

When measuring either BMR or RMR, metabolic chamber size and flowrate should be carefully considered. Because mass-specific metabolic rates are low in resting hummingbirds, a lower flowrate is necessary to achieve oxygen/carbon dioxide measurements that are sufficiently different from baseline to minimize measurement error. A flowrate of 500 mL/min appears to work well for hummingbirds (Powers et al. 2003; Opazo et al. 2005). Small chambers are necessary to reduce the time required to reach an equilibrium value and to better visualize short-term changes in metabolic rate. Chamber volumes of 380–1000 mL have been used successfully for hummingbird species with body weights ranging from 3 to 18 g (Powers et al. 2003; Opazo et al. 2005); however, appropriate chamber size will vary with the body mass of the hummingbird species being studied.

An important consideration when measuring BMR is the requirement that it be measured at night. One of the challenges of measuring BMR in hummingbirds is that they can safely be fasted for only up to a couple of hours before being placed in a metabolism chamber. The authors believe that the best approach is to allow hummingbirds to fill their crops prior to being placed in a metabolism chamber, thus providing them with sufficient energy to enter a resting state (Powers et al. 2003). Furthermore, providing them with a sugar meal will delay torpor (see section 8.4) and optimize chances of recording accurate BMR measurements. With this approach, it is useful to measure both oxygen consumption and carbon dioxide production. This allows for calculation of the respiratory exchange ratio (RER; O_2 consumption/CO_2 production), which explains what the hummingbirds are metabolizing for energy (RER is 1.0 for sugar and approximately 0.7 for protein or fat). When hummingbirds transition to metabolizing protein or fat, they are post-absorptive and ready for measurement of BMR. This procedure can take several hours. At the conclusion of BMR measurement, the authors prefer not to disturb hummingbirds during their resting phase but rather to continue tracking metabolic rate for the remainder of

the night. If birds remain normothermic, they can be returned to a holding cage. However, birds should be allowed to feed for a period before being returned to the dark. Alternatively, they can be left in place with the lights on for the remainder of the night.

Approaches to measurement of RMR vary depending on what the researcher is attempting to measure. For example, if the goal is to measure perching metabolic rate, a perch is included inside the chamber and measurements are made in the light. If the aim is to measure the metabolic cost of being alert, measurements can be made under BMR conditions but during the day. Regardless of the measurement conditions, hummingbirds must be given approximately 1–2 h to acclimate to the chamber. The most common error in measuring metabolic rate is impatience.

8.4. Measuring Torpor Metabolic Rate

Measuring nighttime metabolic rate—including torpor metabolism— in captive hummingbirds can be accomplished by using the open-flow respirometry methods described above. Protocol design depends on the specific topic(s) being addressed in the study. Classic studies investigating the effects of temperature on metabolic rates during torpor have used standard open-flow system configurations (primarily positive pressure) similar to those described above (Hainsworth and Wolf 1970, 1972, 1978; Wolf et al. 1972; Krüger et al. 1982; Hiebert 1990; Bucher and Chappell 1992, 1997). Because the metabolic rate during torpor is extremely low, flowrates as low as 150 mL/min have been used successfully used to separate metabolic response from background values (Bucher and Chappell 1997). However, emerging evidence indicates that torpor metabolism during scripted experiments may differ from ways in which torpor is used by hummingbirds subjected to natural light and temperature conditions (Shankar et al. 2020b).

Several studies that have addressed the ecological importance of torpor in energy management have used protocols that measured metabolic parameters under semi-natural conditions of temperature and photoperiod (Hiebert 1991; Bech et al. 1997; Powers et al. 2003; Shankar et al. 2020b). Some studies have refrained from trapping hummingbirds until the end of the day to allow for normal daytime energy acquisition and endogenous energy storage and to allow birds to fill their crops prior to measurement (Powers et al. 2003; Shankar et al. 2020b). To allow for completely natural temperature and light cycles, Shanker et al. (2020b) placed metabolism chambers outdoors and used a negative pressure, open-flow design. Feeder bases containing sucrose solution were placed in metabolism chambers to allow hummingbirds to feed either at the start or end of the metabolic trial. Inclusion of the feeder base necessitated a larger chamber (approximately 7 L) but the researchers used a flowrate of 500 mL/min, which allowed for measurement of equilibrium metabolic values in under 30 min. This approach appears to have yielded evidence of shallow torpor use, a novel pattern of hypothermia for hummingbirds, and is worth considering if appropriate for the questions being asked.

8.5. Hovering Metabolic and Evaporation Rates

Hovering metabolic rate (HMR) and respiratory evaporative water loss (REWL) can be measured using a negative pressure system attached to a mask as described above. Mask respirometry has been used in a variety of studies involving measurement of HMR (Bartholomew and Lighton 1986; Chai et al. 1998; Welch et al. 2007; Welch and Altshuler 2008; Powers et al. 2012; Groom et al. 2018). Mask-based respirometry has been well-described in the literature (Welch, Jr. 2011). Mask respirometry can be used to make metabolic measurements both in the field (Powers et al. 2012; Groom et al. 2018) or in the laboratory, including during wind tunnel studies (Clark and Dudley 2010; Powers et al. 2012, 2015; Sapir and Dudley 2012). Wind tunnel studies allow metabolic measurements during both forward and backward flight (Sapir and Dudley 2012).

With mask respirometry, the mask is attached to a feeder so that the hummingbird must insert its head into the mask to feed. While the bird is feeding, the negative pressure system pulls respiratory gases exhaled by the hummingbird from the mask, which are then directed to open-flow analyzers. The mask should be sufficiently large to allow a hummingbird to comfortably insert its entire head while not obstructing shoulder movement. If the mask is too long, hummingbirds will have to overextend to reach the feeder and might attempt to

grab the edge of the mask with their feet. The mask itself can be constructed of anything cylindrical and transparent; syringe barrels work well. Syringes also make good feeders for mask respirometry because they are small and masks can be easily attached. The authors recommend angling the mask down slightly, which allows the hummingbird to easily extend its head into the mask while hovering normally. Regardless of the feeder/mask combination, it is important to prevent the sugar solution from dripping into the mask, which can cause substantial errors in measurement of respiratory gases. Because HMR can be over 10 times that of BMR (Suarez 1992; Suarez and Moyes 1992), higher flowrates can be used and are necessary to capture all exhaled respiratory gases. The minimum recommend flowrate for measurement of HMR is 1500 mL/min in relatively still air.

Unlike during measurement of BMR, RMR, and torpor, respiratory gas measurements made during mask respirometry are not equilibrium values and therefore cannot be used to directly calculate HMR (Bartholomew and Lighton 1986). Instead, total volume of oxygen consumed, carbon dioxide produced, or respiratory water evaporated must be calculated by integrating the area under the metabolic response curve. Analyses should be performed only during feeding bouts that are at least three seconds long. Once the total volume of respiratory gas is calculated, it can be divided by the time the hummingbird's head was in the mask to calculate HMR or REWL. If a feeding bout involves multiple head insertions, the time of each insertion must be summed. Two methods have been used to do this (Bartholomew and Lighton 1986; Welch and Suarez 2006). The method used most often commonly employs photoresistors, which—when occluded—mark the presence of the hummingbird's head in the mask. Alternatively, video recordings can be used to time feeding visits (Powers et al. 2012).

8.6. Measuring Field Metabolic Rate

Field metabolic rate (FMR) is a measurement of the energetic cost of animals living in the wild and is often used to estimate daily energy expenditure (DEE). The optimal method for measuring FMR is the doubly labeled water (DLW) method, which allows direct measurement of carbon dioxide production in free-living animals (Nagy 1983; Speakman 1997). In the DLW method, carbon dioxide production is measured by marking an animal's body water pool with isotopes. A small amount of water containing a high amount of both deuterium (^{2}H) and oxygen-18 (^{18}O) is injected into the bird (i.e., the "body water pool") and the rate at which these isotopes are eliminated over time (the turnover rate) is measured. During this process, ^{18}O is lost from the hummingbird's body water pool as both carbon dioxide and water, whereas ^{2}H is lost primarily as water. As such, the difference in turnover rate between the two isotopes is assumed to be due to carbon dioxide production. Both ^{18}O and ^{2}H are stable isotopes, so no special permitting is required. A detailed description of the DLW method has been published (Speakman 1997). Understanding this basic theory is important, particularly as described in Chapter 17 of the cited text.

Although the DLW method works very well for measuring FMR, it is a challenging technique to use on hummingbirds. The primary reason that isotope turnover rate is typically measured using blood samples and hummingbirds have a limited blood volume that can be safely sampled. To date, only three published studies have used this method on hummingbirds (Powers and Nagy 1988; Weathers and Stiles 1989; Powers and Conley 1994). However, current methods for measuring isotopic enrichment require very small samples of body water so that use of the DLW method on hummingbirds is now more feasible than it previously was.

To use the DLW method, hummingbirds are trapped as described elsewhere in this review. Birds are then weighed to the nearest 0.01 g, and a small amount of isotopic water (approximately 2 µL/g) is injected into the muscle. The precise volume may vary depending on the isotope enrichment of the solution. Depending on the species, the isotopic water should be injected using a 50- or 100-µL precision glass syringe (Hamilton Company, Reno, NV) that was tared previously. Prior to injection, the syringe and its contents should be weighed to the nearest 0.0001 g. This is important because the isotopic solution will have a higher mole mass than non-isotopic water, so conversion of injection volume to injection mass for calculation of carbon dioxide production is not trivial. It is important not to inject more than the indicated volume of isotopic water. Today's isotope analyzers are actually better at

detecting relatively lower versus higher isotope enrichment. The current expense of ^{18}O provides additional motivation to conserve.

After injection, hummingbirds should be allowed to rest quietly while the injected isotopic water equilibrates with their body water pool. This usually requires approximately 45 min (DRP laboratory) (Shankar et al. 2019). During this equilibration period, hummingbirds are placed in a mesh bag and distanced from human activity. After equilibration, the initial blood sample should be collected in a heparinized microcapillary tube as described elsewhere in this review (see section 4.2). A minimum of 15 µL of blood (approximately 10 µL of water) is generally sufficient for use in the LGR Liquid Water Isotope Analyzer ® (Los Gatos Research, San Jose, CA), but the amount of blood required may differ depending on the instrument used. After blood collection, the capillary tube ideally should be flame-sealed and refrigerated. A small butane jeweler's torch works well for sealing capillary tubes and is easily used in the field. In addition to study hummingbirds, blood should be collected as described above from a minimum of three injected hummingbirds for each species being studied. These samples will serve as background values required in the calculation of carbon dioxide production. Following blood collection, the researcher should ensure that the collection site has stopped bleeding. The hummingbird is then hand fed a 20–25% sucrose solution to provide energy prior to release. The bird sampled also must be marked for recapture identification. If work is being performed at a site where hummingbirds are banded, band numbers work well. Otherwise, color marking can be employed. Use of newly inserted RFID tags is not recommended because they may impact normal behavior during the release period. Once released, hummingbirds can then engage in their normal daytime/nighttime energy use patterns.

One of the biggest challenges of the DLW method is recapturing injected birds one day later. Water turnover rates are high enough in endothermic animals that body water returns to background levels after two days or less; therefore, recapture after being free-living for 24 h after release is important. However, the recapture rate is only about 10% in most studies, although this rate is highly variable. Hummingbirds appear to have good memories, and when researchers are using modified Russell traps, it is often difficult to trap the same birds two days in a row. An alternative trapping method, such as mist nets, can be used on the second day. Once a hummingbird is recaptured, it must be reweighed and a second blood sample must be collected. Afterwards, the hummingbird can be fed and released.

Because of the difficulty in getting blood samples from hummingbirds, it is important to understand that the DLW method can work using any type of body water sample just as long as the initial and final samples derive from the same source. DLW studies have successfully used urine samples, which can be relatively easy to obtain given the high-water turnover rate of hummingbirds. Urine collection is best done in the morning (see section 7). When collecting urine, the sample should be clear and not grossly contaminated with fecal material. Once collected, urine samples are sealed and stored as described above for blood.

Although enrichment of a hummingbird's body water pool is always best done by injecting isotope, feeding isotope in a sugar solution works reasonably well when injection is not allowed. This is because hummingbirds absorb and process most of the water they consume through their kidneys (McWhorter and Rio 1999). Isotope enrichment by feeding is much more involved than by injection and requires longer periods of handling; therefore, this method should only be used as an option of last resort. When using this approach, hummingbirds must be precisely weighed before and after being fed to calculate the amount of enriched nectar that was consumed. When creating the enriched nectar, the sucrose and enriched water must be added on a weight basis. This allows for subtraction of sucrose from the consumed meal by multiplying the weight of the consumed enriched nectar by the proportion of the mass that is enriched water. It is important not to over enrich the hummingbirds. The amount of isotope added to water used to create the enriched nectar will depend on how readily the species of interest takes to hand feeding. The authors use syringes for feeding, but any type of feeder will work. Note that when using this method, it is much harder to control how much isotope the hummingbirds receive because there is no way to accurately measure consumption.

9. ORGAN TISSUE SAMPLING FROM HUMMINGBIRD SPECIMENS

9.1. Trends and Applications

Literature review yielded 39 unique studies that demonstrated trends in organs sampled, quantities extracted, and applications (Table 9). Some of the research applications for sampling tissues include phylogenetics (Gerwin and Zink 1998; Chaves and Smith 2011; McGuire et al. 2014; Weinstein et al. 2014), epidemiology (Magagna et al. 2019), toxicology (Godoy et al. 2014; Filigenzi et al. 2019; Graves et al. 2019), and physiology (Mathieu-Costello et al. 1992). Due to their small body size and a paucity of techniques for antemortem tissue sampling, carcasses are most commonly utilized for tissue samples. Fresh tissue samples are sometimes needed for certain types of modern genomic testing, such as transcriptome and high-throughput sequencing. However, many studies do not require fresh specimens, so older frozen specimens can be used instead of collecting new specimens. As stated previously, museums are an ideal starting point for obtaining samples because museum curators are often associated with local communities that provide birds for archiving purposes. Alternatively, academic institutions or research programs that archive specimens for future studies may be considered. Other options include licensed wildlife rehabilitators or wildlife rehabilitation centers, which may have birds that were euthanized or did not survive the rehabilitation process.

Whether tissue is sampled from live birds or harvested from deceased individuals, quantities of available tissue can be less than optimal for analysis. Therefore, analytic methods may require modification to adjust for small sample volumes.

9.2. Detailed Methods for Sampling and Storing Organs Harvested from Hummingbird Specimens

Assuming a whole bird has been procured, the protocol for harvesting may vary depending on the organs to be extracted. However, some general guidelines can prove helpful. If the bird is to be vouchered as a museum specimen, the following method for gaining access to organs is necessary in order to preserve the carcass. The specimen should be placed on its back with the ventral surface exposed. The specimen weight, measured in grams, should be recorded before the bird is sampled. The breast feathers are parted and a #15 or #10 scalpel blade is used to make an incision from the mid-furculum caudally along the keel. The skin and muscle overlying the abdominal area is then incised to expose the coelomic cavity. Lateral to the keel on either side, the ribs are transected from the level of the abdomen cranial to the coracoid bones. The keel is then elevated to expose the internal organs.

If the specimen is not going to be archived, the following dissection technique can be used to obtain access to the organs for sampling. The feathers are parted and an incision is made with a scalpel from the lateral commissure of the beak on either side, to the ventral midline, at the junction of the neck and head, then distally along the midline, over the keel, to the cloaca. The skin can then be bluntly dissected and pulled away from the keel and pectoral muscles bilaterally, exposing most of the pectoral muscles. Then an incision can be made on each side of the keel, through the ribs and through the clavicle, bilaterally, allowing the entire breast plate (keel, pectoral muscles, and ribs) to be elevated and moved to the side, exposing the coelomic cavity.

Once the organs are accessible, tweezers or small forceps can be used to isolate the desired organ while small surgical scissors are used to dissect it from the lining of the body cavity. The organ can then be transferred to storage. Tools should be soaked for 5 min in a 1% sodium hypochlorite solution and rinsed with distilled water before and between sample collection for purposes of cleaning and minimizing DNA contamination.

Given the wide range of organ functions and associated specific research applications, there may be organ- or application-specific methods for storage and analysis (Table 9). For example, for genomic banking, all tissues of interest can be stored in the same cryotube. However, other research designs might require that no cross contamination of tissues occur, necessitating storage of tissues in separate vials.

Table 9. Summary of usage and storage conditions for tissue samples collected from hummingbirds as reported in published studies.[a]

Tissue	Count	Amount	Applications	Storage[†]	References
Brain	9	Not reported	Pathology, physiology	Formalin or cryoprotected in 30% sucrose or immersion fixed in 4% paraformaldehyde in 0.1 M phosphate buffer or embedded in gelatin	Iwaniuk and Wylie 2007; Iwaniuk et al. 2009; Godoy et al. 2013; Reiser et al. 2013; González-Gómez et al. 2014a, b; Gaede et al. 2019; Magagna et al. 2019; Diao et al. 2020
Heart	9	26–31 mg	Energetics, genetics, pathology, physiology	Formalin or frozen in liquid nitrogen and then at -70° C or dried at 60° C for 6 days	Gerwin and Zink 1989, 1998; López-Calleja and Bozinovic 2003; López-Calleja et al. 2003; Cortés-Rodríguez et al. 2008; Godoy et al. 2013; Reiser et al. 2013; Magagna et al. 2019; Diao et al. 2020
Intestine	8	21–24 mg	Energetics, gut bacteria, pathology, physiology	Formalin or frozen in liquid nitrogen and then at -80° C or dried at 60° C for 6 days	López-Calleja and Bozinovic 2003; López-Calleja et al. 2003; Preest et al. 2003; Zamparo et al. 2003; Williams et al. 2012; Godoy et al. 2013; Reiser et al. 2013; Magagna et al. 2019
Kidney	8	3–5 mg	Energetics, pathology, physiology	Formalin or dried at 60° C for 6 days	Casotti et al. 1998; Beuchat et. al 1999; López-Calleja and Bozinovic 2003; López-Calleja et al. 2003; Godoy et al. 2013; Reiser et al. 2013; Mikoni et al. 2017; Magagna et al. 2019
Liver	8	Not reported	Energetics, genetics, pathology, physiology	Formalin or frozen in liquid nitrogen and then at -70° C or dried at 60° C for 6 days	López-Calleja and Bozinovic 2003; López-Calleja et al. 2003; Cortés-Rodríguez et al. 2008; Godoy et al. 2013; Mikoni et al. 2017; Lim et al. 2019; Magagna et al. 2019; Diao et al. 2020
Lungs	4	16–20 mg (dry)	Energetics, pathology, physiology	Formalin or frozen in liquid nitrogen and then at -80° C or dried at 60° C for 6 days	López-Calleja and Bozinovic 2003; Williams et al. 2012; Godoy et al. 2013; Magagna et al. 2019
Pectoral muscle	10	Not reported	Genetics, physiology	Frozen or RNAlater or ethanol or salt extraction of genomic DNA	Mathieu-Costello et al. 1992; Gerwin and Zink 1998; Lance et al. 2009; Bailey et al. 2013; Reiser et al. 2013; Benham and Witt 2016; Prosdocimi et al. 2016; Mikoni et al. 2017; Magagna et al. 2019; Diao et al. 2020
Other muscle	13	Not reported	Genetics, pathology, physiology	Formalin or frozen in liquid nitrogen and then at -80°C to -20°C or dried at 60°C for 6 days	Gerwin and Zink 1989; Cortés-Rodríguez et al. 2008; Welch et al. 2009; Fernández et al. 2011a, b; Oyler-McCance et al. 2011; Donovan et al. 2013; Godoy et al. 2013; Reiser et al. 2013; Velten and Welch Jr 2014; Benham et al. 2015; Lim et al. 2019; Magagna et al. 2019

Table 9. (cont.)

Tissue	Count	Amount	Applications	Storage[†]	References
Carcass	3	Variable	Toxicology, Micro CT imaging	Frozen -80°C	Filigenzi et al. 2019; Graves et al. 2019; Riede and Olson 2020
Syrinx	1	Variable	Micro CT imaging	Fixed tissue - 0.1 M phosphate-buffered saline (PBS) in solution with 0.05% sodium acid	Monte et al. 2020
Tissue (not specified)	1	Not reported	Genetics	Museum specimens	Hernandez-Banos et al. 2020

[a] Literature searched until September 2020.

[†]Tissues fixed in formalin should be transferred to paraffin-embedded blocks as soon as possible (ideally within 2–3 days) for optimal preservation of RNA/DNA for later molecular testing by immunohistochemistry, in situ hybridization, or polymerase chain reaction.

10. MUSCLE SAMPLING FROM HUMMINGBIRD SPECIMENS

10.1. Trends and Applications

A literature review yielded 23 records of hummingbird muscle samples being collected from specimens. The vast majority of these records reported sampling of the pectoral muscle (Table 9).

10.2. Detailed Methods for Muscle Sampling from Hummingbird Specimens

10.2.1. Dissection.—Using dissection techniques and a size #15 or #10 scalpel blade, the entire deep and superficial pectoral muscles can be harvested from both sides of the keel.

10.2.2. Punch Biopsy.— The authors have found that a 6-mm dermal biopsy punch (Miltex®, Inc., Princeton, New Jersey) is extremely useful for obtaining pectoral muscle samples while limiting damage to the surrounding tissue. This method is recommended if the specimen is to be vouchered as a museum specimen. In addition to inflicting minimal damage on the specimen, this method allows for immediate harvesting of tissue irrespective of the need to prepare a museum specimen. Soaking the biopsy punch in 1% hypochlorite solution for 5 min and rinsing with distilled water is recommended before and between sampling. The specimen also can be refrozen for later preparation as a study skin.

To obtain a muscle sample, the breast feathers are parted with water or 95% ethyl alcohol, exposing the skin and underlying breast muscle. The biopsy punch is pressed downward and rotated so that the circular blade of the punch penetrates the pectoral muscle. The instrument is then rolled back and forth between the thumb and index finger to rotate the blade. This motion can be continued until the metal tip of the biopsy punch contacts the keel. A 6-mm diameter punch allows for a substantial muscle sample to be obtained from hummingbirds while still facilitating tissue extraction from the instrument and leaving an acceptable defect size for a museum specimen. Although a 4 mm diameter punch still provides an adequate sample and produces a smaller defect, extraction of the sample from the core of the instrument is more challenging. If the carcass is to be used as a study skin, a small plug of cotton can be placed in the muscle defect to protect the feathers from body fluids. Note that the efficacy of dermal punch muscle sampling depends, in part, on the degree of carcass autolysis that has occurred prior to sampling. Autolysis can soften tissue and complicate sample extraction from the instrument.

10.3. Muscle Specimen Storage and Analysis

The precise methods used to store muscle tissue depend on the research application. The authors found 10 records in the literature that used pectoral muscle samples for a range of purposes (Table 9). Most modern museums store muscle samples in cryovials in a -80° C freezer or liquid nitrogen tissue bank.

11. ASSESSING BIRDS FOR REHABILITATION OR EUTHANASIA, METHODS OF EUTHANASIA, AND POST-MORTEM EXAMINATION

11.1. Trends and Applications

A literature review revealed 17 publications in which hummingbirds were euthanized or lethal take was a component of the study and the euthanasia method was reported. Numerous manuscripts did not report the method of euthanasia (n=28). In those that did, the most frequently used method was carbon dioxide asphyxiation, followed by overdose of ketamine/xylazine, along with reports of cervical dislocation and anesthesia followed by either nitrogen asphyxiation or ketamine/xylazine overdose (Table 10).

If a hummingbird is injured and has the potential to recover, it should be taken to a permitted wildlife rehabilitator, which can range from a private individual to a center. When an injured hummingbird is deemed non-releasable (most commonly because of a broken

Table 10. Summary methods used for euthanasia or lethal take in published studies involving hummingbirds as study subjects.[a,b]

Method	Count	References
Anesthesia followed by nitrogen asphyxiation	1	Reiser et al. 2013
Anesthesia followed by ketamine/xylazine overdose	1	Donovan et al. 2013
CO_2 asphyxiation	3	Casotti et al. 1998; Beuchat et. al 1999; Preest et al. 2003
Cervical dislocation	2	López-Calleja and Bozinovic 2003; López-Calleja et al. 2003
Sodium pentobarbital	1	Mathieu-Costello et al. 1992
Thoracic (cardiac) compression alone	3	Fernández et al. 2011a, b; Fernandez and Suarez 2011
Ketamine/xylazine overdose without anesthesia	5	Welch Jr and Altshuler 2009; Reiser et al. 2013; González-Gómez et al. 2014a, b; Gaede et al. 2019
Isoflurane followed by asphyxiation	1	Myrka and Welch 2017

[a] Literature searched until September 2020.

[b] Numerous references reported bird euthanasia or lethal take (n=28) without reporting the technique.

wing) or if a bird is extremely ill, euthanasia may be necessary to relieve pain and suffering. Guidelines for the humane euthanasia of animals have been established by the American Veterinary Medical Association (AVMA) (Leary et al. 2020). These guidelines are required to be updated every 10 years but interim revisions can be made. In order to ensure that researchers are following the most current guidelines, the AVMA resources web page should be consulted regularly (https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals).

Depending on the situation and individual bird, an indirect method of euthanasia (e.g., carbon dioxide overdose) versus an active method (e.g., cervical dislocation) might be less traumatic for the person euthanizing the animal. Some IACUCs have minimum requirements for basic training in euthanasia methods. At minimum, individuals who are hummingbird research site leaders must be trained in active euthanasia methods if they will be employing them at their site. Some institutional IACUCs also require training in indirect methods of euthanasia. Special considerations for euthanasia of wildlife, as opposed to captive animals, have been outlined previously (Paul et al. 2016; Engilis, Jr. et al. 2018). A quick death is preferable to inducing additional distress by capturing, manipulating, and transporting an animal to a facility where euthanasia can be performed. Unacceptable methods of euthanasia include freezing (ice crystallization of tissues is very painful), suffocation, vehicle exhaust, or incorrect application of an approved method. Before working in the field, the investigator or lead individual for the team should decide how euthanasia will be conducted if such action is necessary. If the chosen method requires special agents or equipment, those—in addition to a stethoscope—should be essential parts of the field equipment. If the euthanasia method does not require a chemical or gas agent and will be imposed physically, the BBL permittee or sub-permittee must be trained prior to being allowed to use the method in the field. Euthanizing a hummingbird can be traumatic for the individual performing the euthanasia and/or for team members; therefore, pre-emptive conversations can be helpful.

After a bird has died, tissue samples can be collected, the bird can be frozen and subsequently prepared as a museum specimen, or the animal can be fixed in 10% formalin and submitted for histopathology for disease assessment and/or cause of death. Most state and federal permits require dead birds to be deposited in an accredited museum to ensure long-term research value.

11.2. Assessing a Hummingbird's Condition: Administering First Aid

When assessing the need to conduct first aid procedures, the welfare of the hummingbird takes priority. Individuals working in the field should learn basic first aid procedures to treat minor injuries, such as minor lacerations that occasionally result from birds being captured in mist nets. If BBL permittees have received training in more complex first aid treatments for birds, they can conduct those procedures under a BBL permit as long as the bird is either released or transferred to a rehabilitator within 24 hr. If birds require first aid procedures that are beyond the training of a BBL permittee, the individual must transfer the bird to a veterinarian or rehabilitator within 24 hr to receive appropriate care. First aid treatment by USFWS Migratory Bird Scientific Collecting permittees is not authorized unless the permittee is a veterinarian or permitted wildlife rehabilitator or the permit includes authorization for first aid and treatment of minor injuries (50 CFR § 21.12, 50 CFR § 21.31). USFWS permit applicants can request authorization to perform first aid and treat minor injuries by including a description of their training and experience along with their permit application. If a scientist has both BBL and USFWS Migratory Bird Scientific Collecting permits, administration of first aid procedures requires first aid and minor injury treatment authorization on at least one permit.

11.3. Assessing Hummingbird Health: Rehabilitation or Euthanasia

If a hummingbird is injured during banding or tagging activities and the extent of the injury leads the BBL permittee to believe that euthanasia is the only appropriate option, euthanasia can be performed by the individual holding a BBL master or sub-permit. Guidelines for assessing whether a bird should be euthanized are outlined later in this section. The BBL permit does not provide general authorization to euthanize birds and should not be used to euthanize a healthy bird. If a hummingbird is injured during banding activities and has the potential for recovery, it should be transferred to a licensed wildlife rehabilitator for treatment.

If a hummingbird has a pre-existing injury or illness and is captured with the intent of banding, tagging, or sampling the bird (i.e., birds that are captured for banding and/or sampling but then are discovered to be injured before the procedure), a BBL permit authorizes permittees to evaluate the condition of the bird and either euthanize it or take it to a licensed wildlife rehabilitator or veterinarian for treatment. In cases of captured birds with pre-existing injuries, the BBL permittee must first determine whether the bird should be released in its current condition. If it appears to be otherwise healthy and/or able to survive with the pre-existing condition, the BBL permittee can release the bird back to the wild with or without further treatment. If the BBL permittee decides to release the bird, the individual must decide whether or not to first band the bird. Banding birds with pox lesions anywhere on the body is not advised because lesions can spread to the distal legs and feet. The BBL does not have a stated policy for banding birds with a single foot, and the decision is left up to the BBL permittee (B. Peterjohn, pers. comm., 28 June 2020). From a welfare perspective, birds with a single foot should not be banded on the remaining leg. Free ranging hummingbirds with one leg have been known to survive; therefore, this pre-existing condition might warrant releasing the bird versus taking it to a wildlife rehabilitator. Similarly, birds with pre-existing beak injuries should be released unless the bird's condition warrants taking it to a veterinarian or a wildlife rehabilitator authorized to determine if the bird should be rehabilitated or euthanized (50 CFR § 21.12, 50 CFR § 21.31).

If an injured hummingbird is found by a BBL permittee out of context of capturing the bird for banding or if someone brings an injured hummingbird to a BBL permittee, the BBL permittee is allowed to conduct limited activities to promote the bird's welfare. An example would be allowing a window-strike bird to recover in isolation. However, if the bird requires first-aid treatment before it can be released, it must be taken to a permitted wildlife rehabilitator or veterinarian for appropriate care (50 CFR § 21.12, 50 CFR § 21.31).

If a hummingbird is injured during research activities authorized under a USFWS Migratory Bird Scientific Collecting permit, the researcher should take the bird to a licensed rehabilitator or veterinarian for treatment (50 CFR § 21.12 and 50 CFR § 21.31) if lethal take is not authorized on their permit. Alterna-

tively, a researcher could proactively request collection authorization as part of their USFWS Migratory Bird Scientific Collecting permit application (CFR § 21.23). Demonstration of training and/or experience with lethal take and/or euthanasia techniques will be required for permit authorization. Specifically, if a researcher only possesses a USFWS Migratory Bird Scientific Collecting permit and lethal take of the species is authorized on the permit, the researcher can euthanize the bird. However, if the researcher only has a USFWS Migratory Bird Scientific Collecting permit and lethal take of that species is not authorized on the permit, the individual must take the bird to a licensed rehabilitator or veterinarian for assessment in accordance with USFWS Migratory Bird Rehabilitation regulations (50 CFR § 21.31). In that instance, the researcher is not allowed to euthanize the bird. USFWS regulations allow veterinarians to temporarily care for and stabilize wild birds without a permit (50 CFR § 21.12). As mentioned previously, first aid or minor injury treatment is allowed by a USFWS permittee such treatment is authorized on the permit; however, medical treatment beyond the training of the permittee is not authorized unless the permittee is a veterinarian or permitted wildlife rehabilitator (50 CFR § 21.12, 50 CFR § 21.31).

If a hummingbird becomes moribund in the field while a bander and/or researcher is working with it, the bird should be warmed and offered small amounts of sugar water before euthanasia is considered. Because hummingbirds live on the energetic edge, they may appear to be unrecoverable; however, they often recover after receiving minimal supportive care. Euthanasia is the most humane option for birds that sustain compound wing fractures (i.e., injuries in which a fractured bone penetrates the skin) or neck fractures during the course of a banding or sampling event. Hummingbirds that do not have a wing droop but cannot hover due to soft tissue wing injuries should be considered for rehabilitation. If given sufficient time (sometimes months), these birds may improve to the point of being releasable.

If a hummingbird has a significant injury that will prevent its release and the injury was pre-existing or occurred during a banding/sampling event, placement in a zoologic institution or an organization that accepts non-releasable wildlife might be a consideration. Such placement requires approval from the local USFWS Regional Migratory Bird Permit Office. If the animal is non-releasable, there are no placement options, and/or the bird would endure distress and discomfort while being held captive, euthanasia is the most humane option.

Wildlife rehabilitators or veterinarians are authorized to treat injuries that occur during a banding or sampling activity and are experienced in determining whether a bird should be rehabilitated or euthanized for pre-existing conditions (50 CFR § 21.12, 50 CFR § 21.31). Avian veterinarians are best equipped to work with hummingbirds, and they can be found at https://www.humanesociety.org/resources/how-find-wildlife-rehabilitator. In addition, wildlife rehabilitators can be found by state at https://www.humanesociety.org/resources/how-find-wildlife-rehabilitator. The following USFWS regulations (50 CFR § 21.31, https://www.ecfr.gov/cgi-bin/text-idx?SID=0e9fd773e2a078e4c54d159ce4a9be80&node=50:9.0.1.1.4.3.1.11&rgn=div8) stipulate which conditions necessitate a wildlife rehabilitator to euthanize a bird:

> You must euthanize any bird that cannot feed itself, perch upright, or ambulate without inflicting additional injuries to itself where medical and/or rehabilitative care will not reverse such conditions. You must euthanize any bird that is completely blind, and any bird that has sustained injuries that would require amputation of a leg, a foot, or a wing at the elbow or above (humero-ulnar joint) rather than performing such surgery, unless: (A) A licensed veterinarian submits a written recommendation that the bird should be kept alive, including an analysis of why the bird is not expected to experience the injuries and/or ailments that typically occur in birds with these injuries and a commitment (from the veterinarian) to provide medical care for the bird for the duration of its life, including complete examinations at least once a year; (B) A placement is available for the bird with a person or facility authorized to possess it, where it will receive the veterinary care described in paragraph (e)(4)(iii)(A) of the USFWS regulations; (C) The issuing office specifically authorizes continued possession, medical treatment, and rehabilitative care of the bird.

11.4. Methods for Euthanizing a Hummingbird Due to Injury or Illness

11.4.1. Overdose of inhaled anesthetic agent (Isoflurane).—Overdose of an inhaled anesthetic can be the sole euthanasia method or the first step in a two-step process (anesthesia followed by euthanasia). For hummingbirds, anesthesia overdose is best accomplished by soaking a cotton ball with isoflurane liquid and placing it in a small container with the bird. The bird should be left in the container until respirations have ceased for at least three continuous minutes. Disadvantages of this method include the logistics required to possess and maintain a Food and Drug Administration (FDA) controlled substance and the risk of human exposure to concentrations above 2 ppm within 1 hr (according to the National Institute for Occupational Safety and Health [NIOSH]). If individuals are transporting anesthetic agents, such as isoflurane, to field sites in a vehicle, carrying very small volumes (such as two to three isoflurane-soaked cotton balls) in a well-sealed container will minimize accidental human exposure during transport. A bottle of isoflurane, which holds large volumes of the anesthetic, should never be transported inside the cabin of a vehicle where passengers are present. Use of isoflurane is further complicated if air or highway travel to the field site is necessary. Isoflurane is considered a liquid chemical and is not allowed in carry-on or checked luggage. Furthermore, isoflurane is designated by the US Department of Transportation as a class 9 agent, and transport is subject to local, state, and federal regulations. Therefore, despite its advantages in some situations, there may be substantial limitations to use of an inhaled anesthetic in the field.

11.4.2. Overdose of inhaled carbon dioxide (CO_2).—When using an overdose of inhaled CO_2 to euthanize a hummingbird, a compressed gas canister should be used to deliver CO_2 and gradually fill the bird's containment chamber with gas. Gradual (versus rapid) asphyxiation with CO_2 is less likely to be painful for animals, and the bird will ideally be rendered unconscious before gas accumulation causes discomfort. A gradual fill method at a displacement rate 30–70% of the chamber volume per minute is recommended in the AVMA guidelines for euthanasia (Leary et al. 2020). In the field, a small clear container can be used as a gas chamber (Fig. 11). The container should have a very small outlet for oxygen to escape as CO_2 is being introduced. Small portable CO_2 cartridges, a regulator intended for delivery of CO_2 for carbonating beverages or fish tanks, and plastic tubing can be used to deliver CO_2. Dry ice should never be used to deliver CO_2 for euthanasia. Gas should be delivered slowly so the temperature is not drastically reduced inside the container. To determine the CO_2 flow rate, multiply the chamber length, width and height in inches, then divide the product by 61 to determine the volume in liters. Multiply the volume in liters by 30–70% (0.3–0.7) to determine the proper flow rate (liters per minute or LPM). For example, if a chamber is 12 in length x 12 in width x 12 in height the volume is 1728 in or 61 L (1728/61). The proper CO_2 flow rate would be 8 LPM (61 x 0.3) to 20 LPM (61 x 0.7). The bird should remain exposed to CO_2 for at least 3 min after respiration ceases.

Use of CO_2 has many advantages: 1) it is readily available; 2) it is not an FDA-controlled substance; 3) it is effective over a wide range of concentrations; and 4) it poses minimal risk to the person euthanizing a hummingbird when performed properly. Disadvantages to this method are that it may: 1) cause pain; 2) result in air hunger; 3) elicit a fear response if CO_2 is not administered properly; and/or 4) result in artifactual histopathologic pulmonary lesions. In addition, CO_2 accumulation may cause convulsions, which can result in tissue damage and may be distressing to observers. Neonatal hummingbirds may be relatively more tolerant of CO_2 and may require increased concentrations (Leary et al. 2020).

11.4.3. Overdose of pentobarbital anesthetic.—A hummingbird has reduced muscle mass and very limited vascular access relative to the size of the hypodermic needle necessary for delivering pentobarbital solution. Therefore, hummingbirds should be anesthetized with a gas anesthetic prior to injection with pentobarbital. Pentobarbital can be injected into the jugular vein, muscle, or coelomic cavity. Using a Luer lock syringe and needle will minimize the risk of the needle disengaging from the syringe during injection. Intracoelomic delivery seems to be the least stressful method of injection, but the absorption rate is slower. Pentobarbital should not be administered at too high a dose as this will

Figure 11. Illustration of a self-made carbon dioxide chamber for euthanizing a hummingbird in the field. The paper towel separates the hummingbird from the tubing that is piped into the container and minimizes the bird's access to the bottom of the container.

induce severe damage to the lungs, liver, heart, blood vessels, and other tissues, thus interfering with post-mortem examination. Although pentobarbital injection is an effective way to euthanize a bird, pentobarbital is a controlled substance (DEA Schedule II) that can only be stored and distributed by complying with multiple rules and regulations. Therefore, individuals must be vigilant regarding inventory documentation, access by authorized users, multiple individuals auditing the inventory, and locking storage containers.

11.4.4. Rapid cardiac compression (RCC).— Given the small size of a hummingbird, rapid cardiac compression (RCC; previously referred to as thoracic or cardiac/thoracic compression), if performed properly, can quickly euthanize a hummingbird without the need to anesthetize the bird first. However, because RCC is not included in the AVMA euthanasia guidelines as a stand-alone method for euthanasia at the time of this writing, investigators may first be required to render the bird unconscious with an AVMA-approved euthanasia method, such as CO_2 overdose. IACUCs at institutions accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) are required to use AVMA-approved euthanasia methods. However, investigators can request conditional approval for the use of RCC as a stand-alone euthanasia method for hummingbirds citing work published by Engilis (Engilis, Jr. et al. 2018). Before RCC is used in the field, individuals should be trained using a small laboratory bird species and following Engilis' published methods (Engilis, Jr. et al. 2018). For investigators who are not at AALAC-approved schools, RCC as a primary method of euthanasia for hummingbirds could be considered. However, proper training is essential to minimize pain and suffering while the hummingbird is being euthanized.

Using RCC, an individual directly applies pressure to the heart, which results in obstruction of venous return and cessation of cardiac output. In many cases, this results in rapid rupture of the thin-walled regions of the vena cava or atrium and almost instantaneous cessation of brain activity and pulse production (Paul-Murphy et al. 2017). One study (Engilis, Jr. et al. 2018) explains proper application of RCC for euthanasia of birds weighing less than 500 g and describes external cues as a bird progresses toward death. Advantages of this method include: 1) rapid loss of consciousness and death (Paul-Murphy et al. 2017); 2) yield of

specimens in optimal condition for use as specimens for research collections and other purposes; 3) no requirement for drugs or chemicals; and 4) ready application in any field setting, particularly in situations where controlled substances cannot be used (Engilis, Jr. et al. 2018). However, given the small size of a hummingbird, proper placement of fingers below the wings from the dorsal aspect can be difficult, particularly if the researcher has large fingers. As a result, the cardiopulmonary region may not be properly accessed during RCC. If improperly performed, rapid cardiac compression may cause significant suffering before death (Engilis, Jr. et al. 2018).

11.4.5. Cervical dislocation.—Cervical dislocation is an AVMA-approved method of euthanasia (Paul-Murphy et al. 2017) but should be performed only by trained individuals. This method is best facilitated by hanging the hummingbird's head over a well-stabilized edge and applying force between the first cervical vertebrae and the base of the skull to dislocate the head from the neck. Like RCC, there is no need for specialized equipment or anesthetic agent, and if performed properly, cervical dislocation results in instantaneous death. However, this method can be difficult to perform if the researcher is not properly trained. In addition, cervical dislocation does not provide optimal specimens if intact birds are needed. Visual aesthetics of this method can also have significant negative and emotional impacts on observers and operators.

11.4.6. Decapitation.—Decapitation is an AVMA-approved method of euthanasia and is humane if performed properly (Paul-Murphy et al. 2017). The instrument used for decapitation needs to be sharp so that the head is severed in one swift cut and the neck is cut and not crushed. With the right instrument, decapitation will quickly induce loss of consciousness and death. Disadvantages to this method include rendering the specimen useless if the whole bird is needed and the potential for significant negative and emotional effects on observers and operators. Similar to other methods, individuals must be trained in this euthanasia method prior to working in the field.

11.5. Confirmation of Death

For all euthanasia methods, death can be confirmed by cardiac auscultation and pupillary light response. Lack of spontaneous breathing should not be used as a sole criterion for confirming death. A bird near death may exhibit shallow, irregular breathing patterns, which could be interpreted as a lack of spontaneous breathing. In addition, the respiratory patterns of a hummingbird in torpor might be similar to a bird in a moribund state, which could make it difficult to discern the two.

11.5.1. Heartbeat.—Cardiac auscultation is essential to confirm death. Use of a pediatric stethoscope is helpful due to the small size of the instrument's diaphragm, but any stethoscope is acceptable. A stethoscope should be considered essential equipment for individuals doing any work with hummingbirds. Learning to perform cardiac auscultation on a live bird is important prior to using a stethoscope on a dead bird. This will ensure proper use of the stethoscope and provide a reference point. Dual-headed stethoscopes have both bell and diaphragm components. The diaphragm side of the stethoscope's chest piece must be engaged for cardiac auscultation. This is achieved by twisting the chest piece 180 degrees and hearing a "click" as it engages. The user can tap each head to see if the bell or diaphragm component is engaged. Wrapping a thumb under the stethoscope tubing will help keep it from rubbing on other surfaces. This will reduce peripheral noise and allow the user to be more confident that they no longer hear a heartbeat.

11.5.2. Pupillary light response (PLR).—When a very bright light is directed into the eye of a live animal, the pupil will constrict (narrow), indicating a neurologic response. It is essential to use a very bright light (i.e., light emitting diode; LED) when testing the PLR to ensure proper assessment. Upon death, the pupils will not respond to light and instead will become permanently dilated.

11.6. Post-mortem Examination to Determine Cause of Death

If a bird dies unexpectedly or has to be euthanized due to illness or injury, a post-mortem examination (necropsy) is necessary to determine the cause of death. Performing a necropsy after a bird dies suddenly and unexpectedly during handling by a researcher is an important way of contributing to further knowledge. The specimen should be submitted to a veterinarian or

veterinary diagnostic laboratory for necropsy. A list of veterinary diagnostic laboratories and avian veterinarians can be found at https://www.aphis.usda.gov/animal_health/nahln/downloads/all_nahln_lab_list.pdf and https://www.aav.org/search/custom.asp?id=1803, respectively. If an avian veterinarian cannot be found, another veterinarian can be asked to preserve the bird in formalin and submit it to a veterinary pathologist who specializes in exotic birds.

Dead hummingbirds must be kept cold (but not frozen) for up to 72 h after death to minimize autolysis and optimize the post-mortem examination. Freezing causes ice crystallization artifacts in the soft tissues, thus compromising histopathologic evaluation. Ideally, the specimens should be submitted within 24 h of death. The feathers should be moistened with water prior to refrigeration to facilitate rapid chilling of internal tissue. Although autolysis occurs over time, necropsy of a bird that died up to 72 h before submission may still yield a cause of death.

As soon as the specimen is submitted for necropsy, samples for virology, bacteriology, and/or fresh materials for research studies need to be collected. Immediately following sample collection, the specimen should be preserved in 10% buffered formalin by the veterinarian or diagnostic laboratory so that tissues can be prepared for histopathology. Formalin is a carcinogen, and as such, its storage and handling must follow specific requirements from the Occupational Safety and Health Administration (OSHA). Personal protective equipment (e.g., appropriate gloves, eye protection, and face shield) and a well-ventilated space are required when handling formalin. The fixation process will render the specimen unusable for skinning, and the necropsy process significantly damages the carcass, making it less suitable as a museum specimen. If a dead hummingbird is ultimately going to be placed in a museum, necropsy should be avoided.

To properly fix the specimen for necropsy, the operator should wear laboratory grade gloves and spray the bird's external surface with detergent or a dilute disinfectant. This helps reduce feather waterproofing and allows the fixative to penetrate the skin. After locating the keel, a pair of clean scissors is used to incise the skin at the base of the keel. Once the coelomic cavity is entered, the skin is cut along the distal margin of the keel. The skin is then peeled away from the pectoral muscle on either side of the incision. On the lateral aspect of the bird, the ribs are cut at their junction with the keel and the keel is reflected cranially. The clavicle is also transected. Any hemorrhage in the body cavity or gross lesions involving the organs are noted.

The skin on the back of the skull is then reflected. A small cut is made between the skull and the spinal cord on the dorsal surface of the bird, so that the head is only attached to the rest of the body by the front of the skull, skin, and muscles/ligaments. The incision is continued around the skull, making a "skull cap" that can be elevated forward to reveal the brain. Opening the skull ensures that the brain will be properly fixed, but the brain itself should be left in place. The entire specimen should then be placed in a container of formalin, making sure that all the tissue is covered by solution. Placing a paper towel over the specimen helps to keep it submerged. The container is then sealed and clearly labeled. It is important to note that a deceased bird that has been processed for a post-mortem examination will be rendered useless for museum specimen vouchering.

12. Field Collecting Hummingbirds for Specimen-based Research and Museums

Scientific collecting remains an important tool for researchers studying geographic variation, species-level classification, anatomy and morphology, molt and plumage sequences, subspecies or population limits, vouchering geographical records, and biodiversity inventories (Winker et al. 1991; Remsen 1995; Winker 2000; McGuire et al. 2009; Howell 2010; Rocha et al. 2014; Pyle et al. 2015; Clark and Rankin 2020; Puga-Caballero et al. 2020). As mentioned previously, collection of any hummingbird is regulated through state and federal permitting processes, which requires specific scientific collecting permission from the USFWS Migratory Bird Permit Office in the region where the investigator lives. Collecting (live or lethal) permission can be incorporated into federal and state scientific collecting and research permits that authorize

such activities, but it is not a permit condition granted by the BBL. The BBL only authorizes salvage collection (not live or lethal collection) as a permit condition. Permit limits are placed on the number of individual specimens and the species authorized for collection annually.

Two primary methods are used to collect birds: mist nets and small-caliber shotguns. Hummingbird traps described in Section 2.3 also can be used to obtain specimens and might be a better option compared to mist nets or shotguns, depending on the individual's skill level with the latter two options. The proper use of mist nets for capturing live birds is summarized in *The North American Banders' Manual for Hummingbirds* (Russell and Russell 2019). After capture in a mist net, birds can either be immediately euthanized in the net or removed and held in a cloth bird bag prior to euthanasia. Lethal exposure to isoflurane (section 9.4.1) or rapid cardiac compression (section 9.4.4) can be used for euthanasia (Engilis, Jr. et al. 2018). A .22 long rifle or .410 shotgun are best for collecting small birds. The Savage Model 24 .22LR/.410 combination gun (or equivalent) is the preferred gun of choice by field researchers collecting small birds. Shot size is critical to ensure that minimal damage is inflicted on the specimen. For hummingbirds, the .22 caliber long rifle bird shot (25 grain no. 12 shot) is preferred for birds within a 10-m distance. The .410 shotgun should be used for birds 20–30 m away. The .410 shotgun shell should be loaded preferably with number 12 shot but shot size up to number 9 can be used. Do not use the .410 shotgun if the hummingbird is closer than 20 m as the shot can severely damage the specimen.

Many field researchers process their specimens in the field, but specimens also can be processed and frozen for later work in a laboratory setting. However, care of any specimen should begin as soon as it is collected. The collector should have cotton and absorbent dust available. Cotton should be placed inside the mouth, so body fluids do contaminate the feathers, especially if the animal was captured at or near a feeder and the crop is likely to contain sugar water. For specimens collected by gun, cotton also should be used to obstruct the mouth so that blood or ingesta does not contaminate the feathers. However, if the study requires examination of the tongue or beak structure, such as the elastic micropump mechanism of the hummingbird's feeding apparatus (Rico-Guevara and Rubega 2011), the mouth should not be obstructed with cotton. If the bird has been collected by gun, the specimen should be examined for areas of potential blood leakage. Absorbent dust should be placed on those sites to prevent the spread of fluids. After cotton has been placed in the mouth and other fluid leaks are stopped, all feathers should be smoothed on the body, wings, and tail.

Specimens of any type (including salvage) are documented with a collecting data sheet that lists, at a minimum, the location of collection (using descriptive text as well as latitude and longitude data), the date of collection, and the collector's name and affiliation. Many researchers tie a specimen tag of archival paper that contains this basic information on the leg of the specimen. All specimens collected should be recorded in a field journal for reporting and archival purposes. Pencil or archival ink pens should always be used to record data. The Pigma Micron 01, 02, or 03 (Sakura®, San Francisco, CA) are ideal for fieldwork and are acid-free and archival. Ballpoint pens should be avoided because the ink can bleed and smear onto a specimen or data sheet and render the writing illegible, particularly if the specimen is placed in a freezer.

Once the specimen is cleaned and tagged, it should be placed in a pre-made paper cone or rolled in a small tube (Fig. 12A and 12B). Great care should be taken with the beak so it is not damaged during storage. Rolling a hummingbird specimen in a tube containing the data sheet and then securing the tube ends with tape best protects the fragile bill and specimen in a freezer (Fig. 12B). The specimen should then be placed into a sealed plastic bag from which the air has been removed. For long-term storage (up to 6 months), hummingbirds should be kept in a conventional freezer. Frost-free or ultra-cold freezers (i.e., -20° C to -80° C), which cause rapid desiccation of the specimen, should be avoided.

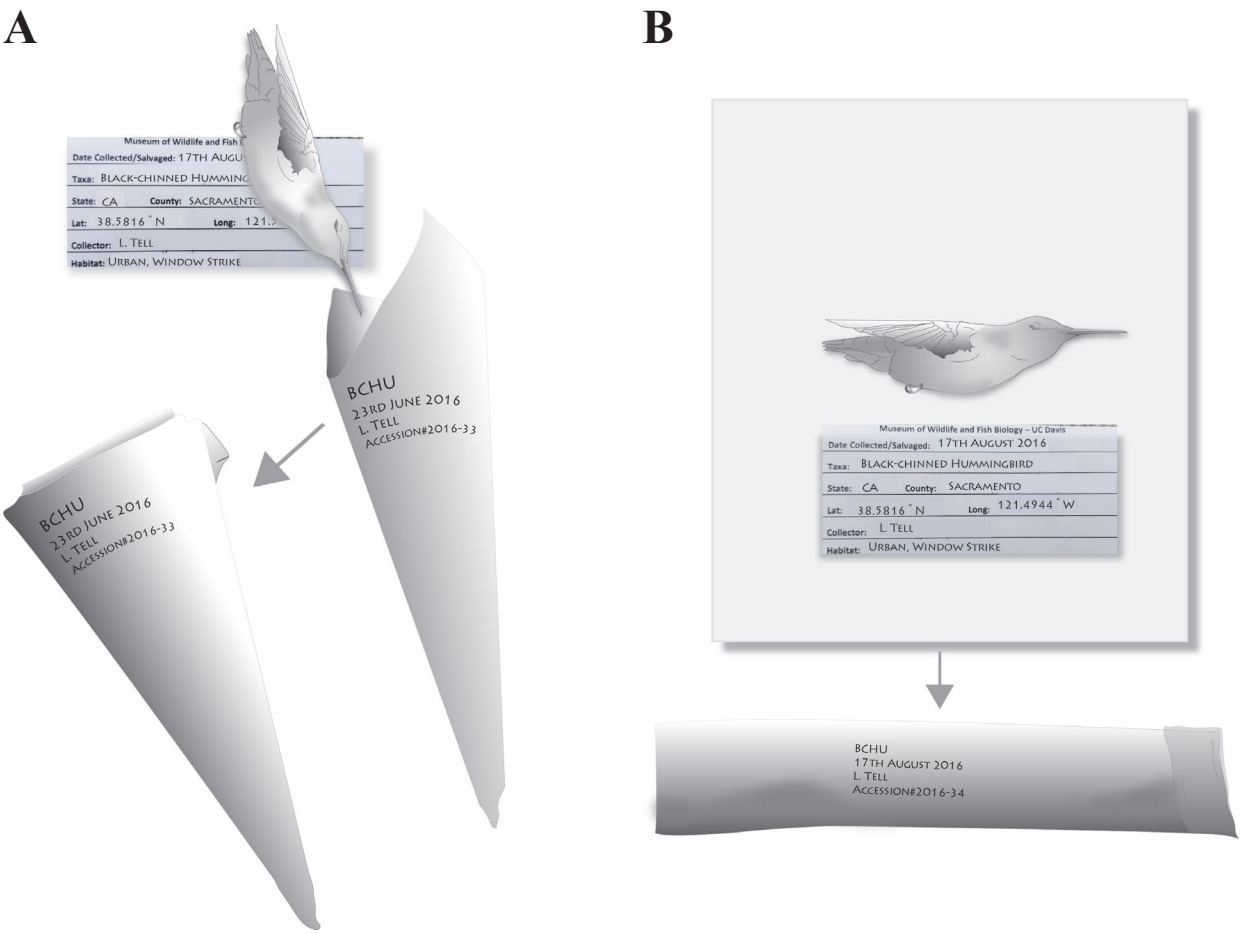

Figure 12. A) Cone method for specimen freezer storage – First recommended method for wrapping hummingbird specimens for freezer archiving. This method will ensure feathers are not damaged and bill and tail protected. Data sheets should always be facing away from bird in case ink is used for recording data on the paper tags. B) Tube method for specimen freezer storage – Second recommended method for wrapping hummingbird specimens for storage in freezer. This method will also ensure feathers are not damaged and bill and tail protected. It is easier to stack specimens using this method compared to cone method. Data sheets should always be facing away from bird in case ink is used for recording data on the paper tags.

13. Balancing Human Safety and Bird Health

When researchers work with live study subjects and collect biological samples, standard operating procedures should be used to avoid disease transmission between live birds and from birds to humans. Researchers should avoid secondary transfer of topical treatments from humans to birds (e.g., sunscreen, insecticide, or related products) and should be aware that their shoes, clothing, and bodies (especially their respiratory tract and hands) can be fomites. Even if all birds in a work session appeared healthy, equipment should be cleaned afterward. Recommended protocols include cleaning equipment with a mild disinfectant, washing bird holding bags with a dilute (10%) sodium hypochlorite (bleach) solution, and thoroughly rinsing any equipment and/or holding bags with water after disinfection. Indirect transmission of avian pox can occur whenever hummingbirds are held and sampled. In addition, virus can be transferred by contaminated materials through a break in the skin. Therefore, after working with a bird potentially infected with pox, any

instruments or materials that came in contact with the bird should be set aside for disinfection. Any scab material shed from lesions on the bird should also be cleaned from the work surface. Pox virus is highly resistant to drying and can survive for months to years. A 10% sodium hypochlorite solution prepared within 4 h before use is optimal for decontaminating surfaces, instruments, and bird holding bags. Important considerations for using sodium hypochlorite as a disinfectant are detailed by the World Health Organization (WHO 2014). Sodium hypochlorite germicidal cleaners and wipes are also made by various manufacturers. Although they have not been tested for decreasing the transmission of avian pox, they could potentially be used to reduce contamination.

Zoonotic diseases (diseases that spread between animals and humans) are a key consideration when obtaining samples from hummingbirds. To the authors' knowledge, zoonotic disease transmission has not been documented between hummingbirds and humans. West Nile virus has been detected in hummingbird tissue samples but an insect vector is required for transmission to humans. Regardless, prevention of disease transmission by conducting best practices should be a priority for any fieldwork. These practices include good hand hygiene, no consumption of drinks/food in the work area, no co-washing of feeders with human dishes, no recapping of hypodermic needles, and disposing of needles and/or syringes directly into a laboratory-grade sharps container or a sharps container approved for medical use. If a bird goes into respiratory arrest, human mouth-to-beak assisted ventilation should not be performed. Even though diseases such as avian influenza have not been reported in hummingbirds, people should limit risks of exposure.

When working with hummingbirds, bees and wasps are another concern due to their attraction to sugar water. Therefore, team members may experience stings, which—in some cases—may result in severe, even life-threatening allergic reactions (anaphylaxis) requiring emergency treatment. As a precaution, all team members should have an antihistamine, such as diphenhydramine, available. Although antihistamines are available over the counter, individuals should be responsible for bringing their own medications that have been procured for their personal use. Knowing the location of the nearest emergency hospital is also integral to an emergency action plan.

CONCLUSION

This review is intended to serve as a guide for scientists interested in pursuing or expanding research involving hummingbirds, with an emphasis on welfare issues that are unique to these birds. The authors present general considerations and baseline practices that are important when working with live hummingbirds as study subjects. As research involving this unique family of birds grows, guidelines for minimizing subject risk and optimizing animal welfare while furthering scientific methods should be constantly reviewed and expanded.

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DISCLAIMER

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LITERATURE CITED

Abrahamczyk, S., and S. S. Renner. 2015. The temporal build-up of hummingbird/plant mutualisms in North America and temperate South America. BMC Evolutionary Biology 15:104. https://doi.org/10.1186/s12862-015-0388-z

Altshuler, D. L., P. Chai, and J. S. P. Chen. 2001. Hovering performance of hummingbirds in hyperoxic gas mixtures. Journal of Experimental Biology 204:2021–2027.

AWA. 1966. Animal Welfare Act. https://www.nal.usda.gov/awic/animal-welfare-act-public-law-89-544-act-august-24-1966. Accessed 26 September 2020.

AWA. 1970. Animal Welfare Act. https://www.nal.usda.gov/awic/public-law-91-579-animal-welfare-act-amendments-1970. Accessed 26 September 2020.

AWAR. 2020. Animal Welfare Act and Regulations. https://www.aphis.usda.gov/animal_welfare/downloads/AC_BlueBook_AWA_508_comp_version.pdf. Accessed 26 September 2020.

Backus, L., L. Foss, and L. A. Tell. 2019. West Nile virus in hummingbirds in California, USA, 2005–17. Journal of Wildlife Diseases 55:903–907.

Baek, H. E., R. R. Bandivadekar, P. Pandit, M. Mah, R. N. Sehgal, and L. A. Tell. 2020. TaqMan quantitative real-time PCR for detecting Avipoxvirus DNA in various sample types from hummingbirds. PLOS ONE 15(6):e0230701. https://doi.org/10.1371/journal.pone.0230701

Bailey, I. E., G. Segelbacher, S. D. Healy, T. A. Hurly, and J. M. Pemberton. 2013. Microsatellite variation in Rufous Hummingbirds (*Selasphorus rufus*) and evidence for a weakly structured population. Journal of Ornithology 154:1029–1037.

Bakken, B. H., T. J. McWhorter, E. Tsahar, and C. M. del Rio. 2004. Hummingbirds arrest their kidneys at night: diel variation in glomerular filtration rate in *Selasphorus platycercus*. Journal of Experimental Biology 207:4383–4391.

Bakken, B. H., and P. Sabat. 2006. Gastrointestinal and renal responses to water intake in the green-backed firecrown (*Sephanoides sephanoides*), a South American hummingbird. American Journal of Physiology. Regulatory Integrative and Comparative Physiology 291:R830–R836.

Bakken, B. H., and P. Sabat. 2007. Evaporative water loss and dehydration during the night in hummingbirds. Revista Chilena de Historia Natural 80:267–273.

Baltosser, W. H. 1978. New and modfied methods for colour-marking hummingbirds. Bird-Banding 49:47–49.

Bandivadekar, R. R., P. S. Pandit, R. Sollmann, M. J. Thomas, S. M. Logan, J. C. Brown, A. P. Klimley, and L. A. Tell. 2018. Use of RFID technology to characterize feeder visitations and contact network of hummingbirds in urban habitats. PLOS ONE 13(12):e0208057. https://doi.org/10.1371/journal.pone.0208057

Barbachano-Guerrero, A., A. A. Vásquez-Aguilar, A. A. Aguirre, A. A. Zavala-Norzagaray, E. C. Gonzalez, A. L. Terrazas, and J. L. Aguilar-Faisal. 2019. West Nile virus prevalence in wild birds from Mexico. Journal of Wildlife Diseases 55:425–431.

Bartholomew, G. A., T. R. Howell, and T. J. Cade. 1957. Torpidity in the white-throated swift, Anna hummingbird, and poor-will. Condor 59:145–155.

Bartholomew, G. A., and J. R. Lighton. 1986. Oxygen consumption during hover-feeding in free-ranging Anna hummingbirds. Journal of Experimental Biology 123:191–199.

Bassett, F., and D. Cubie. 2009. Wintering hummingbirds in Alabama and Florida: species diversity, sex and age ratios, and site fidelity. Journal of Field Ornithology 80:154–162.

Bech, C., A. S. Abe, J. F. Steffensen, M. Berger, and J. Bicudo. 1997. Torpor in three species of Brazilian hummingbirds under semi-natural conditions. Condor 99:780–788.

Benham, P. M., A. M. Cuervo, J. A. McGuire, and C. C. Witt. 2015. Biogeography of the Andean metaltail hummingbirds: contrasting evolutionary histories of tree line and habitat-generalist clades. Journal of Biogeography 42:763–777.

Benham, P. M., and C. C. Witt. 2016. The dual role of Andean topography in primary divergence: functional and neutral variation among populations of the hummingbird, *Metallura tyrianthina*. BMC Evolutionary Biology 16:22. https://doi.org/10.1186/s12862-016-0595-2

Berger, M. 1974a. The energetics of hovering hummingbirds at altitude conditions. Journal of Ornithology 115:273–288.

Berger, M. 1974b. Oxygen-consumption and power of hovering hummingbirds at varying barometric and oxygen pressures. Naturwissenschaften 61:407.

Berger, M., and J. S. Hart. 1972. Respiration in the hummingbird *Amazilia fimbriata* during hovering at different ambient temperatures. Journal of Comparative Physiology 81:363–380.

Beuchat, C. A., M. R. Preest, and E. J. Braun. 1999. Glomerular and medullary architecture in the kidney of Anna's Hummingbird. Journal of Morphology 240:95–100.

Bishop, C. A., A. J. Moran, M. C. Toshack, E. Elle, F. Maisonneuve, and J. E. Elliott. 2018. Hummingbirds and bumble bees exposed to neonicotinoid and organophosphate insecticides in the Fraser Valley, British Columbia, Canada. Environmental Toxicology and Chemistry 37:2143–2152.

Bishop, C. A., M. B. Woundneh, F. Maisonneuve, J. Common, J. E. Elliott, and A. J. Moran. 2020. Determination of neonicotinoids and butenolide residues in avian and insect pollinators and their ambient environment in Western Canada (2017, 2018). Science of the Total Environment 737. https://doi.org/10.1016/j.scitotenv.2020.139386

Bradshaw, A. C., L. A. Tell, H. B. Ernest, S. Bahan, J. Carlson, and R. N. M. Sehgal. 2017. Detection and prevalence of *Haemoproteus archilochus* (Haemosporida, Haemoproteidae) in two species of California hummingbirds. Parasitology Research 116:1879–1885.

Brewer, L. W., C. A. Redmond, J. M. Stafford, and G. E. Hatch. 2011. Marking Ruby-throated Hummingbirds with radio frequency identification tags. Journal of Wildlife Management 75:1664–1667.

Bridge, E. S. 2004. The effects of intense wing molt on diving in alcids and potential influences on the evolution of molt patterns. Journal of Experimental Biology 207:3003–3014.

Brown, C., F. Torres, and R. Rech. 2012. A field manual for collection of specimens to enhance diagnosis of animal diseases. Boca Publications Group Incorporated, Boca Raton, Florida.

Bucher, T. L., and M. A. Chappell. 1992. Ventilatory and metabolic dynamics during entry into and arousal from torpor in selasphorus hummingbirds. Physiological Zoology 65:978–993.

Bucher, T. L., and M. A. Chappell. 1997. Respiratory exchange and ventilation during nocturnal torpor in hummingbirds. Physiological Zoology 70:45–52.

Calder III, W. A., N. M. Waser, S. M. Hiebert, D. W. Inouye, and S. Miller. 1983. Site-fidelity, longevity, and population dynamics of broad-tailed hummingbirds: a ten year study. Oecologia 56:359–364.

Calder, W. A. 1994. When do hummingbirds use torpor in nature? Physiological Zoology 67:1051–1076.

Carpenter, F. L. 1974. Torpor in an Andean hummingbird: its ecological significance. Science 183:545–547.

Carpenter, F. L., and R. E. MacMillen. 1976. Energetic cost of feeding territories in an Hawaiian honeycreeper. Oecologia 26:213–223.

Casotti, G., C. A. Beuchat, and E. J. Braun. 1998. Morphology of the kidney in a nectarivorous bird, the Anna's hummingbird *Calypte anna*. Journal of Zoology 244:175–184.

Céspedes, L. N., L. I. Pavan, J. A. Hazlehurst, and J. E. Jankowski. 2019. The behavior and diet of the Shining Sunbeam (*Aglaeactis cupripennis*): a territorial high-elevation hummingbird. The Wilson Journal of Ornithology 131:24–34.

Chai, P., A. C. Chang, and R. Dudley. 1998. Flight thermogenesis and energy conservation in hovering hummingbirds. Journal of Experimental Biology 201:963–968.

Chai, P., and R. Dudley. 1996. Limits to flight energetics of hummingbirds hovering in hypodense and hypoxic gas mixtures. Journal of Experimental Biology 199:2285–2295.

Chai, P., R. Harrykissoon, and R. Dudley. 1996. Hummingbird hovering performance in hyperoxic heliox: effects of body mass and sex. Journal of Experimental Biology 199:2745–2755.

Chaui-Berlinck, J. G., J. E. P. W. Bicudo, L. H. A. Monteiro, and C. A. Navas. 2002. Oscillatory pattern in oxygen consumption of hummingbirds. Journal of Thermal Biology 27:371–379.

ChauiBerlinck, J. G., and O. Bicudo. 1995. Unusual metabolic shifts in fasting hummingbirds. Auk 112:774–778.

Chaves, J. A., J. P. Pollinger, T. B. Smith, and G. LeBuhn. 2007. The role of geography and ecology in shaping the phylogeography of the speckled hummingbird (*Adelomyia melanogenys*) in Ecuador. Molecular Phylogenetics and Evolution 43:795–807.

Chaves, J. A., and T. B. Smith. 2011. Evolutionary patterns of diversification in the Andean hummingbird genus

Adelomyia. Molecular Phylogenetics and Evolution 60:207–218.

Chaves, J. A., J. T. Weir, and T. B. Smith. 2011. Diversification in *Adelomyia* hummingbirds follows Andean uplift. Molecular Ecology 20:4564–4576.

Chavez-Zichinelli, C. A., L. Gomez, R. Ortiz-Pulido, C. Lara, R. Valdez, and M. C. Romano. 2014. Testosterone levels in feces predict risk-sensitive foraging in hummingbirds. Journal of Avian Biology 45:501–506.

Chen, C. C. W., and K. C. Welch, Jr. 2014. Hummingbirds can fuel expensive hovering flight completely with either exogenous glucose or fructose. Functional Ecology 28:589–600.

Clark, C. J. 2011a. Effects of tail length on an escape maneuver of the Red-billed Streamertail. Journal of Ornithology 152:397–408.

Clark, C. J. 2011b. Wing, tail, and vocal contributions to the complex acoustic signals of courting Calliope hummingbirds. Current Zoology 57:187–196.

Clark, C. J. 2014. Harmonic hopping, and both punctuated and gradual evolution of acoustic characters in *Selasphorus* hummingbird tail-feathers. PLOS ONE 9(4):e93829. https://doi.org/10.1371/journal.pone.0093829

Clark, C. J., and R. Dudley. 2010. Hovering and forward flight energetics in Anna's and Allen's hummingbirds. Physiological and Biochemical Zoology 83:654–662.

Clark, C. J., D. O. Elias, M. B. Girard, and R. O. Prum. 2013a. Structural resonance and mode of flutter of hummingbird tail feathers. Journal of Experimental Biology 216:3404–3413.

Clark, C. J., D. O. Elias, and R. O. Prum. 2011a. Aeroelastic flutter produces hummingbird feather songs. Science 333:1430–1433.

Clark, C. J., D. O. Elias, and R. O. Prum. 2013b. Hummingbird feather sounds are produced by aeroelastic flutter, not vortex-induced vibration. Journal of Experimental Biology 216:3395–3403.

Clark, C. J., and T. J. Feo. 2008. The Anna's hummingbird chirps with its tail: a new mechanism of sonation in birds. Proceedings of the Royal Society B, Biological Sciences 275:955–962.

Clark, C. J., and T. J. Feo. 2010. Why do *Calypte* hummingbirds "sing" with both their tail and their syrinx? An apparent example of sexual sensory bias. American Naturalist 175:27–37.

Clark, C. J., T. J. Feo, and K. B. Bryan. 2012. Courtship displays and so nations of a hybrid male broad-tailed × black-chinned hummingbird. Condor 114:329–340.

Clark, C. J., T. J. Feo, and I. Escalante. 2011b. Courtship displays and natural history of scintillant (*Selasphorus scintilla*) and volcano (*S. flammula*) hummingbirds. Wilson Journal of Ornithology 123:218–228.

Clark, C. J., J. A. McGuire, E. Bonaccorso, J. S. Berv, and R. O. Prum. 2018a. Complex coevolution of wing, tail, and vocal sounds of courting male bee hummingbirds. Evolution 72:630–646.

Clark, C. J., and E. A. Mistick. 2018a. Kinematic control of male Allen's Hummingbird wing trill over a range of flight speeds. Journal of Experimental Biology 221. http://jeb.biologists.org/lookup/doi/10.1242/jeb.173625

Clark, C. J., and E. A. Mistick. 2018b. Strategic acoustic control of a hummingbird courtship dive. Current Biology 28:1257–1264.

Clark, C. J., and D. Rankin. 2020. Subtle, pervasive genetic correlation between the sexes in the evolution of dimorphic hummingbird tail ornaments. Evolution 74:528–543.

Clark, C. J., D. Rankin, and K. Johnson. 2018b. Female song in Costa's Hummingbird (*Calypte costae*). The Wilson Journal of Ornithology 130:987–992.

Colwell, R. 2011. Leg injuries observed in banded female Anna's Hummingbirds (*Calypte anna*) in central California. North American Bird Banders 36:57–64.

Correa-Lima, A. P. A., I. G. Varassin, N. Barve, and V. P. Zwiener. 2019. Spatio-temporal effects of climate change on the geographical distribution and flowering phenology of hummingbird-pollinated plants. Annals of Botany 124:389–398.

Cortés-Rodríguez, N., B. E. Hernández-Baños, A. G. Navarro-Sigüenza, A. Townsend Peterson, and J. García-Moreno. 2008. Phylogeography and population genetics of the Amethyst-throated Hummingbird (*Lampornis amethystinus*). Molecular Phylogenetics and Evolution 48:1–11.

Cubie, D. 2014. Site fidelity, residency, and sex ratios of wintering Ruby-throated Hummingbirds (*Archilochus colubris*) on the southeastern US Atlantic coast. Wilson Journal of Ornithology 126:775–778.

DeRogatis, A. M., L. V. Nguyen, R. R. Bandivadekar, K. C. Klasing, and L. A. Tell. 2020. Anti-microbial activity of whole blood and plasma collected from Anna's Hummingbirds (*Calypte anna*) against three different microbes. PLOS ONE 15(6):e0234239. https://doi.org/10.1371/journal.pone.0234239

Diao, S. M., R. Poppenga, G. Gonzales Alarcio, J. Foley, R. Bandivadikar, L. Aston, and L. A. Tell. 2020. Concentrations of vitamins A and E in tissue samples from Anna's Hummingbirds (*Calypte anna*): development of an analytical method for measuring vitamin concentrations in small mass tissue samples. Frontiers in Veterinary Science 7:637. https://doi.org/10.3389/fvets.2020.00637

Dick, M. F., A. Alcantara-Tangonan, Y. S. Oghli, and K. C. Welch. 2020. Metabolic partitioning of sucrose and seasonal changes in fat turnover rate in ruby-throated

hummingbirds (*Archilochus colubris*). Journal of Experimental Biology 223. https://jeb.biologists.org/content/223/2/jeb212696

Dittmann, D., and S. Cardiff. 2009. The alternate plumage of the Ruby-throated Hummingbird. Birding 41:32–35.

Dongen, W. F. D. v., I. Lazzoni, H. Winkler, R. A. Vasquez, and C. F. Estades. 2013. Behavioural and genetic interactions between an endangered and a recently-arrived hummingbird. Biological Invasions 15:1155–1168.

Donovan, E. R., B. K. Keeney, E. Kung, S. Makan, J. M. Wild, and D. L. Altshuler. 2013. Muscle activation patterns and motor anatomy of Anna's hummingbirds *Calypte anna* and zebra finches *Taeniopygia guttata*. Physiological and Biochemical Zoology 86:27–46.

Eberts, E. R., M. F. Dick, and K. C. Welch. 2019. Metabolic fates of evening crop-stored sugar in Ruby-throated Hummingbirds (*Archilochus colubris*). Diversity 11:9. https://doi.org/10.3390/d11010009

Eliason, C. M., R. Maia, J. L. Parra, and M. D. Shawkey. 2020. Signal evolution and morphological complexity in hummingbirds (Aves: Trochilidae). Evolution 74:447–458.

Engilis, A., Jr., I. E. Engilis, and J. Paul-Murphy. 2018. Rapid cardiac compression: an effective method of avian euthanasia. Condor 120:617–621.

Epting, R. J. 1980. Functional dependence of the power for hovering on wing disk loading in hummingbirds. Physiological Zoology 53:347–357.

Espín, S., A. Garcıa-Fernández, D. Herzke, R. Shore, B. van Hattum, E. Martınez-López, M. Coeurdassier, I. Eulaers, C. Fritsch, and P. Gómez-Ramırez. 2014. Sampling and contaminant monitoring protocol for raptors. Research Networking Programme-EURAPMON. http://www.eurapmon.net/. Accessed 28 September 2020.

Espín, S., J. Andevski, G. Duke, I. Eulaers, P. Gómez-Ramírez, G.T. Hallgrimsson, B. Helander, D. Herzke, V.L.B. Jaspers, O. Krone, R. Lourenço, P. María-Mojica, E. Martínez-López, R. Mateo, P. Movalli, P. Sánchez-Virosta, R.F. Shore, C. Sonne, N.W. van den Brink, B. van Hattum, A. Vrezec, C. Wernham, and A.J. García-Fernández. 2020. A schematic sampling protocol for contaminant monitoring in raptors. Ambio. https://doi.org/10.1007/s13280-020-01341-9

Espino-Espino, J., F. Rosas, and E. Cuevas-Garcia. 2014. Temporal variation of floral visitors in two sympatric co-flowering *Salvia* species with contrasting pollination syndromes. Revista Mexicana de Biodiversidad 85:161–166.

Evangelista, D., M. J. Fernández, M. S. Berns, A. Hoover, and R. Dudley. 2010. Hovering energetics and thermal balance in Anna's hummingbirds (*Calypte anna*). Physiological and Biochemical Zoology 83:406–413.

Ewald, P. W., and F. L. Carpenter. 1978. Territorial responses to energy manipulations in the Anna hummingbird. Oecologia 31:277–292.

Fair, J. M., and J. Jones. 2010. Guidelines to the use of wild birds in research. Ornithological Council. https://birdnet.org/info-for-ornithologists/guidelines-to-the-use-of-wild-birds-in-research/guidelines-english-3rd-edition-2010/. Accessed 29 September 2020.

Feo, T. J., and C. J. Clark. 2010. The displays and sonations of the black-chinned hummingbird (Trochilidae: *Archilochus alexandri*). Auk 127:787–796.

Fernández, M., F. Bozinovic, and R. Suarez. 2011a. Enzymatic flux capacities in hummingbird flight muscles: a "one size fits all" hypothesis. Canadian Journal of Zoology 89:985–991.

Fernández, M. J., R. Dudley, and F. Bozinovic. 2011b. Comparative energetics of the giant hummingbird (*Patagona gigas*). Physiological and Biochemical Zoology 84:333–340.

Filigenzi, M. S., E. E. Graves, L. A. Tell, K. A. Jelks, and R. H. Poppenga. 2019. Quantitation of neonicotinoid insecticides, plus qualitative screening for other xenobiotics, in small-mass avian tissue samples using UHPLC high-resolution mass spectrometry. Journal of Veterinary Diagnostic Investigation 31:399–407.

Gaede, A. H., C. Gutierrez-Ibanez, M. S. Armstrong, D. L. Altshuler, and D. R. Wylie. 2019. Pretectal projections to the oculomotor cerebellum in hummingbirds (*Calypte anna*), zebra finches (*Taeniopygia guttata*), and pigeons (*Columba livia*). Journal of Comparative Neurology 527:2644–2658.

George, J. W. 2001. The usefulness and limitations of hand-held refractometers in veterinary laboratory medicine: an historical and technical review. Veterinary Clinical Pathology 30:201–210.

Gerwin, J. A., and R. M. Zink. 1989. Phylogenetic patterns in the genus *Heliodoxa* (Aves, Trochilidae): an allozymic perspective. Wilson Bulletin 101:525–544.

Gerwin, J. A., and R. M. Zink. 1998. Phylogenetic patterns in the Trochilidae. Auk 115:105–118.

Gill, F. B. 1988. Trapline foraging by hermit hummingbirds: competition for an undefended, renewable resource. Ecology 69:1933–1942.

Godoy, L. A., L. S. Dalbeck, L. A. Tell, L. W. Woods, R. R. Colwell, B. Robinson, S. M. Wethington, A. Moresco, P. R. Woolcock, and H. B. Ernest. 2013. Characterization of avian poxvirus in Anna's Hummingbird (*Calypte anna*) in California, USA. Journal of Wildlife Diseases 49:978–985.

Godoy, L. A., L. A. Tell, and H. B. Ernest. 2014. Hummingbird health: pathogens and disease conditions in the family Trochilidae. Journal of Ornithology 155:1–12.

Godwin, B. L., M. E. LaCava, B. Mendelsohn, R. B. Gagne, K. D. Gustafson, S. M. L. Stowell, A. Engilis, L. A. Tell, and H. B. Ernest. 2020. Novel hybrid finds a peri-urban

niche: Allen's Hummingbirds in southern California. Conservation Genetics. https://doi.org/10.1007/s10592-020-01303-4

Goldsmith, T. H., and K. M. Goldsmith. 1979. Discrimination of colors by the black-chinned hummingbird, *Archilochus alexandri*. Journal of Comparative Physiology A130:209–220.

Goloff, B. M., and S. Burch. 2012. Stress response in the rufous hummingbird (*Selasphorus rufus*): mechanisms of personality and social dominance. *in* Friday Harbor Laboratories Student Research Papers. https://doi.org/10.1007/s10592-020-01303-4 Accessed 29 September 2020.

González-Gómez, P. L., W. S. Blakeslee, P. Razeto-Barry, R. M. Borthwell, S. M. Hiebert, and J. C. Wingfield. 2014a. Aggression, body condition, and seasonal changes in sex-steroids in four hummingbird species. Journal of Ornithology 155:1017–1025.

González-Gómez, P. L., F. Bozinovic, and R. A. Vásquez. 2011. Elements of episodic-like memory in free-living hummingbirds, energetic consequences. Animal Behaviour 81:1257–1262.

González-Gómez, P. L., N. Madrid-Lopez, J. E. Salazar, R. Suárez, P. Razeto-Barry, J. Mpodozis, F. Bozinovic, and R. A. Vásquez. 2014b. Cognitive ecology in hummingbirds: the role of sexual dimorphism and its anatomical correlates on memory. PLOS ONE 9(3):e90165. https://doi.org/10.1371/journal.pone.0090165

González-Gómez, P. L., P. Razeto-Barry, M. Araya-Salas, and C. F. Estades. 2015. Does environmental heterogeneity promote cognitive abilities? Integrative and Comparative Biology 55:432–443.

González, C., and J. F. Ornelas. 2009. Song variation and persistence of song neighborhoods in a lekking hummingbird. Condor 111:633–640.

González, C., and J. Francisco Ornelas. 2014. Acoustic divergence with gene glow in a lekking hummingbird with complex songs. PLOS ONE 9(10):e109241. https://doi.org/10.1371/journal.pone.0109241

González, C., J. Francisco Ornelas, and C. Gutierrez-Rodriguez. 2011. Selection and geographic isolation influence hummingbird speciation: genetic, acoustic and morphological divergence in the wedge-tailed sabrewing (*Campylopterus curvipennis*). BMC Evolutionary Biology 11:38. https://doi.org/10.1186/1471-2148-11-38

González, O., and B. A. Loiselle. 2016. Species interactions in an Andean bird-flowering plant network: phenology is more important than abundance or morphology. PeerJ 4:e2789. https://doi.org/10.7717/peerj.2789

Graves, E. E., K. A. Jelks, J. E. Foley, M. S. Filigenzi, R. H. Poppenga, H. B. Ernest, R. Melnicoe, and L. A. Tell. 2019. Analysis of insecticide exposure in California hummingbirds using liquid chromatography-mass spectrometry. Environmental Science and Pollution Research 26:15458–15466.

Gregory, T. R., C. B. Andrews, J. A. McGuire, and C. C. Witt. 2009. The smallest avian genomes are found in hummingbirds. Proceedings of the Royal Society B, Biological Sciences 276:3753–3757.

Groom, D. J. E., M. C. B. Toledo, D. R. Powers, B. W. Tobalske, and K. C. Welch, Jr. 2018. Integrating morphology and kinematics in the scaling of hummingbird hovering metabolic rate and efficiency. Proceedings of the Royal Society B, Biological Sciences 28:20172011. http://dx.doi.org/10.1098/rspb.2017.2011

Hadley, A. S., and M. G. Betts. 2009. Tropical deforestation alters hummingbird movement patterns. Biology Letters 5:207–210.

Hagadorn, K. A., L. A. Tell, T. L. Drazenovich, and H. B. Ernest. 2016. Molecular sex identification markers for five North American hummingbird species. Conservation Genetics Resources 8:427–430.

Hainsworth, F. R., B. G. Collins, and L. L. Wolf. 1977. The function of torpor in hummingbirds. Physiological Zoology 50:215–222.

Hainsworth, F. R., and L. L. Wolf. 1970. Regulation of oxygen consumption and body temperature during torpor in a hummingbird, *Eulampis jugularis*. Science 168:368–369.

Hainsworth, F. R., and L. L. Wolf. 1972. Energetics of nectar extraction in a small, high altitude, tropical hummingbird, *Selasphorus flammula*. Journal of Comparative Physiology 80:377–387.

Hainsworth, F. R., and L. L. Wolf. 1976. Nectar characteristics and food selection by hummingbirds. Oecologia 25:101–113.

Hainsworth, F. R., and L. L. Wolf. 1978. Regulation of metabolism during torpor in temperate zone hummingbirds. Auk 95:197–199.

Hardesty, J. L., and K. C. Fraser. 2010. Using deuterium to examine altitudinal migration by Andean birds. Journal of Field Ornithology 81:83–91.

Harrigan, R. J., R. Sedano, A. C. Chasar, J. A. Chaves, J. T. Nguyen, A. Whitaker, and T. B. Smith. 2014. New host and lineage diversity of avian haemosporidia in the northern Andes. Evolutionary Applications 7:799–811.

Hayashi, S., and T. Sakaguchi. 1975. Capillary tube urinalysis for small animals. Laboratory Animal Science 25:781–782.

Hazlehurst, J. A., and J. O. Karubian. 2018. Impacts of nectar robbing on the foraging ecology of a territorial hummingbird. Behavioural Processes 149:27–34.

Henning, J. D., L. DeGroote, and C. R. Dahlin. 2015. Implementation of a sampling strategy to detect West Nile virus in oral and cloacal samples in live song birds. Journal of Virological Methods 222:81–84.

Hernandez-Banos, B. E., L. Zamuduo-Beltrán, and B. Mila. 2020. Phylogenetic relationships and systematics of a subclade of Mesoamerican emerald hummingbirds (Aves: Trochilidae: Trochilini). Zootaxa 4748:581–591.

Hernández-Soto, M., Y. Licona-Vera, C. Lara, and J. F. Ornelas. 2018. Molecular and climate data reveal expansion and genetic differentiation of Mexican Violet-ear *Colibri thalassinus thalassinus* (Aves: Trochilidae) populations separated by the Isthmus of Tehuantepec. Journal of Ornithology 159:687–702.

Hiebert, S. 1993a. Seasonal-changes in body-mass and use of torpor in a migratory hummingbird. Auk 110:787–797.

Hiebert, S. M. 1990. Energy costs and temporal organization of torpor in the Rufous Hummingbird (*Selasphorus rufus*). Physiological Zoology 63:1082–1097.

Hiebert, S. M. 1991. Seasonal differences in the response of Rufous Hummingbirds to food restriction: body mass and the use of torpor. Condor 93:526–537.

Hiebert, S. M. 1992. Time-dependent thresholds for torpor initiation in the rufous hummingbird (*Selasphorus rufus*). Journal of Comparative Physiology B 162:249–255.

Hiebert, S. M. 1993b. Seasonality of daily torpor in a migratory hummingbird. The Auk:110:787–797.

Hiebert, S. M., M. Ramenofsky, K. Salvante, J. C. Wingfield, and C. L. Gass. 2000a. Noninvasive methods for measuring and manipulating corticosterone in hummingbirds. General and Comparative Endocrinology 120:235–247.

Hiebert, S. M., K. Salvante, M. Ramenofsky, and J. C. Wingfield. 2000b. Seasonal differences in the response of corticosterone and nocturnal torpor to energy challenge in rufous hummingbirds. American Zoologist 40:1055–1055.

Hiebert, S. M., K. G. Salvante, M. Ramenofsky, and J. C. Wingfield. 2000c. Corticosterone and nocturnal torpor in the rufous hummingbird (*Selasphorus rufus*). General and Comparative Endocrinology 120:220–234.

Hilton, B., Jr., and M. W. Miller. 2003. Annual survival and recruitment in a Ruby-throated Hummingbird population, excluding the effect of transient individuals. Condor 105:54–62.

Hou, L., M. Verdirame, and K. C. Welch. 2015. Automated tracking of wild hummingbird mass and energetics over multiple time scales using radio frequency identification (RFID) technology. Journal of Avian Biology 46:1–8.

Howell, S. N. 2002. Hummingbirds of North America: the photographic guide. Christopher Helm Publishers Limited. London, United Kingdom.

Howell, S. N. 2010. Molt in North American birds. Houghton Mifflin Harcourt Publishing Company, New York.

Hurly, T. A., S. Franz, and S. D. Healy. 2010. Do rufous hummingbirds (*Selasphorus rufus*) use visual beacons? Animal Cognition 13:377–383.

Ibarra, V., M. Araya-Salas, Y.-p. Tang, C. Park, A. Hyde, T. F. Wright, and W. Tang. 2015. An RFID based smart feeder for hummingbirds. Sensors 15:31751–31761.

Inouye, D. W., and A. D. McGuire. 1991. Effects of snowpack on timing and abundance of flowering in *Delphinium nelsonii* (Ranunculaceae): implications for climate change. American Journal of Botany 78:997–1001.

Iwaniuk, A. N., H. Marzban, J. M. P. Pakan, M. Watanabe, R. Hawkes, and D. R. W. Wylie. 2009. Compartmentation of the cerebellar cortex of hummingbirds (Aves: Trochilidae) revealed by the expression of zebrin II and phospholipase Cβ4. Journal of Chemical Neuroanatomy 37:55–63.

Iwaniuk, A. N., and D. R. W. Wylie. 2007. Neural specialization for hovering in hummingbirds: hypertrophy of the pretectal nucleus lentiformis mesencephali. Journal of Comparative Neurology 500:211–221.

Kapoor, J. A. 2012. Improved methods for color-marking hummingbirds. Journal of Field Ornithology 83:186–191.

Kim, E. J., M. Wolf, V. M. Ortega-Jimenez, S. H. Cheng, and R. Dudley. 2014. Hovering performance of Anna's hummingbirds (*Calypte anna*) in ground effect. Journal of the Royal Society Interface 11:20140505. https://doi.org/10.1098/rsif.2014.0505

King, A., and J. McLelland. 1984. Birds: their structure and function, 2nd ed. Balliere, Tindall, London.

Krüger, K., R. Prinzinger, and K. L. Schuchmann. 1982. Torpor and metabolism in hummingbirds. Comparative Biochemistry and Physiology Part A Physiology 73:679–689.

Lance, S. L., C. Hagen, T. C. Glenn, R. T. Brumfield, K. F. Stryjewski, and G. R. Graves. 2009. Fifteen polymorphic microsatellite loci from Jamaican streamertail hummingbirds (*Trochilus*). Conservation Genetics 10:1195–1198.

Lasiewski, R. C. 1963a. The energetic cost of small size in hummingbirds. Proceedings International Ornithological Congress 13:1095–1103.

Lasiewski, R. C. 1963b. Oxygen consumption of torpid, resting, active and flying hummingbirds. Physiological Zoology 36:122–140.

Lasiewski, R. C., and R. J. Lasiewski. 1967. Physiological responses of the Blue-throated and Rivoli's hummingbirds. Auk 84:34–48.

Lasiewski, R. C., W. W. Weathers, and M. H. Bernstein. 1967. Physiological responses of the giant hummingbird, *Patagona gigas*. Comparative Biochemistry and Physiology 23:797–813.

Leary, S., W. Underwood, R. Anthony, S. Cartner, T. Grandin, C. Greenacre, S. Gwaltney-Brant, M. A. McCrackin, R. Meye, D. Miller, J. Shearer, T. Turner, and R. Yanong. 2020. American Veterinary Medical Association guidelines for the euthanasia of animals. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf. Accessed 28 September 2020.

Lee, C., L. A. Tell, T. Hilfer, and R. L. Vannette. 2019. Microbial communities in hummingbird feeders are distinct from floral nectar and influenced by bird visitation. Proceedings of the Royal Society B 286:20182295. https://doi.org/10.1098/rspb.2018.2295

Licona-Vera, Y., and J. Francisco Ornelas. 2014. Genetic, ecological and morphological divergence between populations of the endangered Mexican Sheartail Hummingbird (*Doricha eliza*). PLOS ONE 9(7):e101870. https://doi.org/10.1371/journal.pone.0101870

Lighton, J. 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, Inc., New York.

Lim, M. C., C. C. Witt, C. H. Graham, and L. M. Dávalos. 2019. Parallel molecular evolution in pathways, genes, and sites in high-elevation hummingbirds revealed by comparative transcriptomics. Genome Biology and Evolution 11:1573–1585.

López-Calleja, M. V., and F. Bozinovic. 1995. Maximum metabolic rate, thermal insulation and aerobic scope in a small-sized Chilean hummingbird (*Sephanoides sephanoides*). Auk 112:1034–1036.

López-Calleja, M. V., and F. Bozinovic. 2003. Dynamic energy and time budgets in hummingbirds: a study in *Sephanoides sephaniodes*. Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology 134:283–295.

López-Calleja, M. V., M. J. Fernández, and F. Bozinovic. 2003. The integration of energy and nitrogen balance in the hummingbird *Sephanoides sephaniodes*. Journal of Experimental Biology 206:3349–3359.

Lotz, C. N., and C. Martínez Del Rio. 2004. The ability of rufous hummingbirds *Selasphorus rufus* to dilute and concentrate urine. Journal of Avian Biology 35:54–62.

Lumeij, J. 1987. Avian clinical pathology. General considerations. Veterinary Quarterly 9:249–254.

Magagna, M., E. Noland, L. A. Tell, G. Purdin, B. Rideout, M. W. Lipman, and D. Agnew. 2019. Histopathologic findings in free-ranging California hummingbirds, 1996–2017. Journal of Wildlife Diseases 55:343–351.

Maglianesi, M. A., N. BlüThgen, K. BöHning-Gaese, and M. Schleuning. 2014a. Morphological traits determine specialization and resource use in plant-hummingbird networks in the neotropics. Ecology 95:3325–3334.

Maglianesi, M. A., K. Bohning-Gaese, and M. Schleuning. 2014b. Different foraging preferences of hummingbirds on artificial and natural flowers reveal mechanisms structuring plant-pollinator interactions. Journal of Animal Ecology 84:655–664.

Malpica, A., and J. F. Ornelas. 2014. Postglacial northward expansion and genetic differentiation between migratory and sedentary populations of the broad-tailed hummingbird (*Selasphorus platycercus*). Molecular Ecology 23:435–452.

Mathieu-Costello, O., R. K. Suarez, and P. W. Hochachka. 1992. Capillary-to-fiber geometry and mitochondrial density in hummingbird flight muscle. Respiration Physiology 89:113–132.

Matta, N. E., I. A. Lotta, G. Valkiunas, A. D. González, M. A. Pacheco, A. A. Escalante, L. I. Moncada, and O. A. Rodriguez-Fandino. 2014. Description of *Leucocytozoon quynzae sp*. nov. (Haemosporida*, Leucocytozoidae*) from hummingbirds, with remarks on distribution and possible vectors of leucocytozoids in South America. Parasitology Research 113:457–468.

McDonald, P. G., and S. C. Griffith. 2011. To pluck or not to pluck: the hidden ethical and scientific costs of relying on feathers as a primary source of DNA. Journal of Avian Biology 42:197–203.

McGuire, J. A., C. C. Witt, J. V. Remsen, Jr., R. Dudley, and D. L. Altshuler. 2009. A higher-level taxonomy for hummingbirds. Journal of Ornithology 150:155–165.

McGuire, J. A., C. C. Witt, J. V. Remsen, Jr., A. Corl, D. L. Rabosky, D. L. Altshuler, and R. Dudley. 2014. Molecular phylogenetics and the diversification of hummingbirds. Current Biology 24:910–916.

McKechnie, A. E., and B. G. Lovegrove. 2002. Avian facultative hypothermic responses: a review. Condor 104:705–724.

McKechnie, A. E., and B. O. Wolf. 2004. The allometry of avian basal metabolic rate: good predictions need good data. Physiological and Biochemical Zoology 77:502–521.

McWhorter, T. J., and C. M. Del Rio. 2000. Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73:313–324.

McWhorter, T. J., D. R. Powers, and C. Martínez Del Rio. 2003. Are hummingbirds facultatively ammonotelic? Nitrogen excretion and requirements as a function of body size. Physiological and Biochemical Zoology 76:731–743.

McWhorter, T. J., C. Martínez del Rio, B. Pinshow, and L. Roxburgh. 2004. Renal function in Palestine Sunbirds: elimination of excess water does not constrain energy intake. Journal of Experimental Biology 207:3391–3398.

McWhorter, T. J., and C. Martínez del Rio. 1999. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? Journal of Experimental Biology 202:2851–2858.

Meadows, M. G., N. I. Morehouse, R. L. Rutowski, J. M. Douglas, and K. J. McGraw. 2011. Quantifying iridescent coloration in animals: a method for improving repeatability. Behavioral Ecology and Sociobiology 65:1317–1327.

Meadows, M. G., T. E. Roudybush, and K. J. McGraw. 2012. Dietary protein level affects iridescent coloration in Anna's

hummingbirds, *Calypte anna*. Journal of Experimental Biology 215:2742–2750.

MBTA (Migratory Bird Treaty Act). 2020. Migratory Bird Treaty Act. U.S. Fish and Wildlife Service, Policies and Regulations, Laws/Legislation. https://www.fws.gov/birds/policies-and-regulations/laws-legislations/migratory-bird-treaty-act.php. Accessed 26 September 2020.

Mikoni, N. A., R. Poppenga, J. T. Ackerman, J. Foley, J. Hazlehurst, G. Purdin, L. Aston, S. Hargrave, K. Jelks, and L. A. Tell. 2017. Trace element contamination in feather and tissue samples from Anna's hummingbirds. Ecological Indicators 80:96–105.

Miller, R. S., and C. L. Gass. 1985. Survivorship in hummingbirds: Is predation important? Auk 102:175–178.

Mironov, S., P. B. Klimov, T. M. Pérez, and B. M. OConnor. 2019. Feather mites of the family Ptyssalgidae (Acari: Analgoidea) associated with hummingbirds and suboscine passerines—A morphological review supplemented by CO1 barcode sequences. Systematic and Applied Acarology 24:1406–1448.

Monte, A., A. F. Cerwenka, B. Ruthensteiner, M. Gahr, and D. N. Düring. 2020. The hummingbird syrinx morphome: a detailed three-dimensional description of the black jacobin's vocal organ. BMC Zoology 5:7. https://bmczool.biomedcentral.com/articles/10.1186/s40850-020-00057-3

Moran, A. J., S. W. Prosser, and J. A. Moran. 2019. DNA metabarcoding allows non-invasive identification of arthropod prey provisioned to nestling Rufous hummingbirds (*Selasphorus rufus*). PeerJ 7:e6586. https://doi.org/10.7717/peerj.6596

Moran, J. A., L. I. Wassenaar, J. C. Finlay, C. Hutcheson, L. A. Isaac, and S. M. Wethington. 2013. An exploration of migratory connectivity of the Rufous Hummingbird (*Selasphorus rufus*), using feather deuterium. Journal of Ornithology 154:423–430.

Mulvihill, R. S., R. C. Leberman, and D. S. Wood. 1992. A possible relationship between reversed sexual dimorphism and reduced male surviviorship in the Ruby-throated Hummingbird. Condor 94:480–489.

Myrka, A. M., and K. C. Welch. 2017. Evidence of high transport and phosphorylation capacity for both glucose and fructose in the ruby-throated hummingbird (*Archilochus colubris*). Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology 224:253–261.

Nagy, K. A. 1983. The doubly labeled water (3HH180) method: a guide to its use. University of California Los Angeles Publication Number 12-1417.

Oniki, Y. 1996. Band sizes of southeastern Brazilian hummingbirds. Journal of Field Ornithology 67:387–391.

Opazo, J. C., M. Soto-Gamboa, and M. J. Fernández. 2005. Cell size and basal metabolic rate in hummingbirds. Revista Chilena de Historia Natural 78:261–265.

Oring, L. W., K. P. Able, D. W. Anderson, L. F. Baptista, J. C. Barlow, A. S. Gaunt, F. B. Gill, and J. C. Wingfield. 1988. Report of committee on use of wild birds in research. Auk 105:1A–41A.

Ornelas, J. F., C. González, A. E. de los Monteros, F. Rodríguez-Gómez, and L. M. García-Feria. 2014. In and out of Mesoamerica: temporal divergence of Amazilia hummingbirds pre-dates the orthodox account of the completion of the Isthmus of Panama. Journal of Biogeography 41:168–181.

Ornelas, J. F., C. González, B. E. Hernández-Baños, and J. García-Moreno. 2016. Molecular and iridescent feather reflectance data reveal recent genetic diversification and phenotypic differentiation in a cloud forest hummingbird. Ecology and Evolution 6:1104–1127.

Oyler-McCance, S. J., J. A. Fike, T. Talley-Farnham, T. Engelman, and F. Engelman. 2011. Characterization of ten microsatellite loci in the Broad-tailed Hummingbird (*Selasphorus platycercus*). Conservation Genetics Resources 3:351–353.

Parra, J. L. 2010. Color evolution in the hummingbird genus *Coeligena*. Evolution 64:324–335.

Parra, J. L., J. A. McGuire, and C. H. Graham. 2010. Incorporating clade identity in analyses of phylogenetic community structure: an example with hummingbirds. American Naturalist 176:573–587.

Parra, J. L., J. V. Remsen, Jr., M. Alvarez-Rebolledo, and J. A. McGuire. 2009. Molecular phylogenetics of the hummingbird genus *Coeligena*. Molecular Phylogenetics and Evolution 53:425–434.

Paton, D. N. 1910. Creatin excretion in the bird and its significance. Journal Physiology 39:485–504.

Paul-Murphy, J. R., A. Engilis, Jr., P. J. Pascoe, D. C. Williams, K. A. Gustavsen, T. L. Drazenovich, M. K. Keel, T. M. Polley, and I. E. Engilis. 2017. Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*). American Journal of Veterinary Research 78:887–899.

Paul, E., R. S. Sikes, S. J. Beaupre, and J. C. Wingfield. 2016. Animal welfare policy: implementation in the context of wildlife research—policy review and discussion of fundamental issues. Institute for Laboratory Animal Research Journal 56:312–334.

Pavan, L. I., J. E. Jankowski, and J. A. Hazlehurst. 2020. Patterns of territorial space use by Shining Sunbeams (*Aglaeactis cupripennis*), tropical montane hummingbirds. Journal of Field Ornithology 91:1–12.

Pearson, O. P. 1950. The metabolism of hummingbirds. Condor 52:145–152.

PHS (Public Health Service). 2015. Public Health Service policy on humane care and use of laboratory animals. U.S. Department of Health and Human Services, National

Institutes of Health, Office of Laboratory Animal Welfare. NIH Publication No. 15-8013. https://olaw.nih.gov/sites/default/files/PHSPolicyLabAnimals.pdf. Accessed 26 September 2020.

Pieplow, N. 2019. Peterson field guide to bird sounds of western North America. Peterson Field Guides, Houghton Mifflin Harcourt Publishing Company, New York.

Powers, D. R. 1991. Diurnal variation in mass, metabolic rate, and respiratory quotient in Anna's and Costa's Hummingbirds. Physiological Zoology 64:850–870.

Powers, D. R. 1992. Effect of temperature and humidity on evaporative water loss in Anna's hummingbird (*Calypte anna*). Journal of Comparative Physiology B 162:74–84.

Powers, D. R., A. R. Brown, and J. A. Van Hook. 2003. Influence of normal daytime fat deposition on laboratory measurements of torpor use in territorial versus nonterritorial hummingbirds. Physiological and Biochemical Zoology 76:389–397.

Powers, D. R., and T. M. Conley. 1994. Field metabolic rate and food consumption of two sympatric hummingbird species in southeastern Arizona. Condor 96:141–150.

Powers, D. R., P. W. Getsinger, B. W. Tobalske, S. M. Wethington, S. D. Powers, and D. R. Warrick. 2012. Respiratory evaporative water loss during hovering and forward flight in hummingbirds. Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology 161:279–285.

Powers, D. R., and K. A. Nagy. 1988. Field metabolic-rate and food-consumption by free-living Anna's Hummingbirds (*Calypte anna*). Physiological Zoology 61:500–506.

Powers, D. R., B. W. Tobalske, J. K. Wilson, H. Arthur Woods, and K. R. Corder. 2015. Heat dissipation during hovering and forward flight in hummingbirds. Royal Society Open Science 2:150598. https://doi.org/10.1098/rsos.150598

Powers, D. R., J. A. Van Hook, E. A. Sandlin, and T. J. McWhorter. 2010. Arthropod foraging by a southeastern Arizona hummingbird guild. Wilson Journal of Ornithology 122:494–502.

Preest, M. R., and C. A. Beuchat. 1997. Ammonia excretion by hummingbirds. Nature 386:561–562.

Preest, M. R., D. G. Folk, and C. A. Beuchat. 2003. Decomposition of nitrogenous compounds by intestinal bacteria in hummingbirds. Auk 120:1091–1101.

Prinzinger, R., T. Schäfe, and K. L. Schuchmann. 1992. Energy metabolism, respirtory quotient and breathing parameters in two convergent small bird species: the fork-tailed sunbird *Aethopyga christinae* (Nectariniidae) and the Chilean hummingbird *Sephanoides sephanoides* (Trohilidae). Journal of Thermal Biology 17:71–79.

Projecto-Garcia, J., C. Natarajan, H. Moriyama, R. E. Weber, A. Fago, Z. A. Cheviron, R. Dudley, J. A. McGuire, C. C. Witt, and J. F. Storz. 2013. Repeated elevational transitions in hemoglobin function during the evolution of Andean hummingbirds. Proceedings of the National Academy of Sciences of the United States of America 110:20669–20674.

Prosdocimi, F., H. M. Souto, P. A. Ruschi, C. Furtado, and W. B. Jennings. 2016. Complete mitochondrial genome of the versicoloured emerald hummingbird *Amazilia versicolor*, a polymorphic species. Mitochondrial DNA 27:3214–3215.

Puga-Caballero, A., M. del Coro Arizmendi, and L. A. Sánchez-González. 2020. Phylogenetic and phenotypic filtering in hummingbirds from urban environments in Central Mexico. Evolutionary Ecology 34:525–541.

Pyle, P., J. Andrew Engilis, and D. A. Kelt. 2015. Manual for ageing and sexing birds of Bosque Fray Jorge National Park and northcentral Chile, with notes on range and breeding seasonality. Occasional Papers of The Museum of Natural Science, Louisiana State University, Baton Rouge.

Pyle, P. 1997. Identification guide to North American Birds, Part I. Slate Creek Press, Bolinas, California.

Pyle, P., S. N. Howell, and G. M. Yanega. 1997. Molt, retained flight feathers, and age in North American hummingbirds. Pp.155–166 in The era of Allan R. Phillips: a festschrift (R.W. Dickerman, compiler). Horizon Communications, Albuquerque, New Mexico.

Reiser, P. J., K. C. Welch, Jr., R. K. Suarez, and D. L. Altshuler. 2013. Very low force-generating ability and unusually high temperature dependency in hummingbird flight muscle fibers. Journal of Experimental Biology 216:2247–2256.

Remsen, J. 1995. The importance of continued collecting of bird specimens to ornithology and bird conservation. Bird Conservation International 5:146–180.

Rico-Guevara A. and M.A. Rubega. 2011. The hummingbird tongue is a fluid trap, not a capillary tube. Proceedings National Academy of Science United States of America 108:9356–9360.

Riede, T., and C. R. Olson. 2020. The vocal organ of hummingbirds shows convergence with songbirds. Scientific Reports 10:1–14.

Rocha, L. A., A. Aleixo, G. Allen, F. Almeda, C. C. Baldwin, M. V. Barclay, J. M. Bates, A. Bauer, F. Benzoni, and C. Berns. 2014. Specimen collection: an essential tool. Science 344:814–815.

Rodriguez-Gomez, F., and J. Francisco Ornelas. 2014. Genetic divergence of the Mesoamerican azure-crowned hummingbird (*Amazilia cyanocephala*, Trochilidae) across the Motagua-Polochic-Jocotan fault system. Journal of Zoological Systematics and Evolutionary Research 52:142–153.

Rodríguez-Gómez, F., C. Gutiérrez-Rodríguez, and J. F. Ornelas. 2013. Genetic, phenotypic and ecological divergence with gene flow at the isthmus of tehuantepec: the case of the azure-crowned hummingbird (*Amazilia cyanocephala*). Journal of Biogeography 40:1360–1373.

Rodríguez-Gómez, F., and J. F. Ornelas. 2018. Genetic structuring and secondary contact in the white-chested *Amazilia* hummingbird species complex. Journal of Avian Biology 49:jav.01536. https://doi.org/10.1111/jav.01536

Roy, M. S., J. C. Torres-Mura, and F. Hertel. 1998. Evolution and history of hummingbirds (Aves: Trochilidae) from the Juan Fernandez Islands, Chile. Ibis 140:265–273.

Ruf, T., and F. Geiser. 2015. Daily torpor and hibernation in birds and mammals. Biological Reviews 90:891–926.

Russell, S. M., and R. O. Russell. 2019. The North American banders' manual for hummingbirds (J. Pollock and A. Hill, eds.). The North American Banding Council, Point Reyes Station, California

Safra, N., M. M. Christopher, H. B. Ernest, R. Bandivadekar, and L. A. Tell. 2018. Leukocyte reference intervals for free-ranging hummingbirds in Northern California. Journal of Wildlife Diseases 54:607–611.

Sapir, N., and R. Dudley. 2012. Backward flight in hummingbirds employs unique kinematic adjustments and entails low metabolic cost. Journal of Experimental Biology 215:3603–3611.

Schuchmann, K. L. 1979a. Energetical responses to various ambient temperatures in the hummingbird *Ocreatus u. underwoodii* (Energieumsatz in abhängigkeit von der umgebungstemperatur beim Kolibri *Ocreatus u. underwoodii*). Journal of Ornithology 120:311–315.

Schuchmann, K. L. 1979b. Metabolism of flying hummingbirds. Ibis 121:85–86.

Schuchmann, K. L., D. Schmidt-Marloh, and H. Bell. 1979. Energetical regulation of feeding by a tropical hummingbird, *Amazilia tzacatl* (Energtische untersuchungen bei einer tropischen kolibriart, *Amazilia tzacatl*). Journal of Ornithology120:78–85.

Schuchmann, K. L., and D. Schmidtmarloh. 1979a. Metabolic and thermal responses to heat and cold in Streamertail Hummingbirds (*Trochilus polytmus* and *Trochilus scitulus*, Trochilidae). Biotropica 11:123–126.

Schuchmann, K. L., and D. Schmidtmarloh. 1979b. Temperature regulation in non-torpid hummingbirds. Ibis 121:354–356.

Shankar, A., C. H. Graham, J. R. Canepa, S. M. Wethington, and D. R. Powers. 2019. Hummingbirds budget energy flexibly in response to changing resources. Functional Ecology 33:1904–1916.

Shankar, A., D. R. Powers, L. M. Dávalos, and C. H. Graham. 2020a. The allometry of daily energy expenditure in hummingbirds: an energy budget approach. Journal of Animal Ecology 89:1254–1261.

Shankar, A., R. J. Schroeder, S. M. Wethington, C. H. Graham, and D. R. Powers. 2020b. Hummingbird torpor in context: duration, more than temperature, is the key to nighttime energy savings. Journal of Avian Biology 51:e02305. https://doi.org/10.1111/jav.02305

Sieburth, D., and P. Pyle. 2018. Evidence for a prealternate molt-migration in the Rufous Hummingbird and its implications for the evolution of molts in Apodiformes. Auk 135:495–505.

Sierra-Marquez, L., S. Peñuela-Gomez, L. Franco-Espinosa, D. Gomez-Ruiz, J. Diaz-Nieto, J. Sierra-Marquez, and J. Olivero-Verbel. 2018. Mercury levels in birds and small rodents from Las Orquideas National Natural Park, Colombia. Environmental Science and Pollution Research 25:35055–35063.

Snowden, K., B. Daft, and R. W. Nordhausen. 2001. Morphological and molecular characterization of *Encephalitozoon hellem* in hummingbirds. Avian Pathology 30:251–255.

Sosa, J., J. L. Parra, D. G. Stavenga, and M. A. Giraldo. 2020. Sexual dichromatism of the Blue-throated Starfrontlet, *Coeligena helianthea*, hummingbird plumage. Journal of Ornithology 161:289–296.

Speakman, J. 1997. Doubly labelled water: theory and practice. Chapman and Hall, London, United Kingdom.

Stiles, F. G. 1982. Aggressive and courtship displays of the male Anna's Hummingbird. Condor 84:208–225.

Stiles, F. G. 1992. Effects of a severe drought on the population biology of a tropical hummingbird. Ecology 73:1375–1390.

Stiles, F. G., and L. L. Wolf. 1973. Techniques for colour-marking hummingbirds. Condor 75:244–245.

Stiles, F. G., and L. L. Wolf. 1979. Ecology and evolution of lek mating behavior in the Long-tailed Hermit Hummingbird. Ornithological Monographs 27:iii–78.

Suarez, R. K. 1992. Hummingbird flight: sustaining the highest mass-specific metabolic rates among vertebrates. Experientia 48:565–570.

Suarez, R. K., J. R. B. Lighton, G. S. Brown, and O. Mathieu-Costello. 1991. Mitochondrial respiration in hummingbird flight muscles. Proceedings of the National Academy of Sciences of the United States of America 88:4870–4873.

Suarez, R. K., and C. D. Moyes. 1992. Mitochondrial respiration in locust flight muscles. Journal of Experimental Zoology 263:351–355.

Supp, S. R., F. A. La Sorte, T. A. Cormier, M. C. W. Lim, D. R. Powers, S. M. Wethington, S. Goetz, and C. H. Graham. 2015. Citizen-science data provides new insight into annual and seasonal variation in migration patterns. Ecosphere 6:1–19. https://doi.org/10.1890/ES14-00290.1

Swaddle, J. P., and M. S. Witter. 1997. The effects of molt on the flight performance, body mass, and behavior of European starlings (*Sturnus vulgaris*): an experimental approach. Canadian Journal of Zoology 75:1135–1146.

Tello-Ramos, M. C., A. T. Hurly, and S. D. Healy. 2019. From a sequential pattern, temporal adjustments emerge in hummingbird traplining. Integrative Zoology 14:182–192.

Temeles, E. J., and G. A. Bishop. 2019. A hurricane alters pollinator relationships and natural selection on an introduced island plant. Biotropica 51:129–138.

Temeles, E. J., C. R. Koulouris, S. E. Sander, and W. J. Kress. 2009. Effect of flower shape and size on foraging performance and trade-offs in a tropical hummingbird. Ecology 90:1147–1161.

Temeles, E. J., Y. J. Rah, J. Andicoechea, K. L. Byanova, G. S. J. Giller, S. B. Stolk, and W. J. Kress. 2013. Pollinator-mediated selection in a specialized hummingbird-Heliconia system in the Eastern Caribbean. Journal of Evolutionary Biology 26:347–356.

Temeles, E. J., K. C. Shaw, A. U. Kudla, and S. E. Sander. 2006. Traplining by purple-throated carib hummingbirds: behavioral responses to competition and nectar availability. Behavioral Ecology and Sociobiology 61:163–172.

Tiebout, H. M., and K. A. Nagy. 1991. Validation of the doubly labeled water method (HHO)-H-3-0-18 for measuring water flux and CO2 production in the tropical hummingbird *Amazilia saucerottei*. Physiological Zoology 64:362–374.

Tiebout III, H. M. 1991. Daytime energy management by tropical hummingbirds: responses to foraging constraint. Ecology 72:839–851.

Tiebout III, H. M. 1992. Comparative energetics of divergent foraging modes: a doubly labelled water experiment on hummingbird competition. Animal Behaviour 44:895–906.

Trombulak, S. C. 1983. A bathing assembly of Blue-vented Hummingbirds (*Amazilia saucerottei*) in Costa Rica. Condor 85:495–496.

Velten, B. P., and K. C. Welch, Jr. 2014. Myosin heavy-chain isoforms in the flight and leg muscles of hummingbirds and zebra finches. American Journal of Physiology - Regulatory Integrative and Comparative Physiology 306:R845–R851.

Warrick, D. R., B. W. Tobalske, and D. R. Powers. 2005. Aerodynamics of the hovering hummingbird. Nature 435:1094–1097.

Waser, N. M., and W. A. Calder. 1975. Possible impairment of nest-building of hummingbirds by acetate leg tags. Condor 77:361–361.

Weathers, W. W., and F. G. Stiles. 1989. Energetics and water-balance in free-living tropical hummingbirds. Condor 91:324–331.

Weinstein, B. G. 2015. MotionMeerkat: integrating motion video detection and ecological monitoring. Methods in Ecology and Evolution 6:357–362.

Weinstein, B. G., B. Tinoco, J. Luis Parra, L. M. Brown, J. A. McGuire, F. Gary Stiles, and C. H. Graham. 2014. Taxonomic, phylogenetic, and trait beta diversity in South American hummingbirds. American Naturalist 184:211–224.

Welch, K. C., Jr., L. G. M. Herrera, and R. K. Suarez. 2008. Dietary sugar as a direct fuel for flight in the nectarivorous bat *Glossophaga soricina*. Journal of Experimental Biology 211:310–316. Erratum in: 2011. Journal of Experimental Biology 214:3324.

Welch, K. C., Jr. 2011. The power of feeder-mask respirometry as a method for examining hummingbird energetics. Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology 158:276–286.

Welch, K. C., Jr., and D. L. Altshuler. 2009. Fiber type homogeneity of the flight musculature in small birds. Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology 152:324–331.

Welch, K. C., Jr., D. L. Altshuler, and R. K. Suarez. 2007. Oxygen consumption rates in hovering hummingbirds reflect substrate-dependent differences in P/O ratios: carbohydrate as a 'premium fuel'. Journal of Experimental Biology 210:2146–2153.

Welch, K. C., Jr., B. H. Bakken, C. Martínez Del Rio, and R. K. Suarez. 2006. Hummingbirds fuel hovering flight with newly ingested sugar. Physiological and Biochemical Zoology 79:1082–1087.

Welch, K. C., Jr., and R. K. Suarez. 2007. Oxidation rate and turnover of ingested sugar in hovering Anna's (*Calypte anna*) and rufous (*Selasphorus rufus*) hummingbirds. Journal of Experimental Biology 210:2154–2162.

Welch, K.C., Jr., and R. K. Suarez. 2008. Altitude and temperature effects on the energetic cost of hover-feeding in migratory rufous hummingbirds, *Selasphorus rufus*. Canadian Journal of Zoology 86:161–169.

Wells, D. J. 1993a. Ecological correlates of hovering flight of hummingbirds. Journal of Experimental Biology 178:59–70.

Wells, D. J. 1993b. Muscle performance in hovering hummingbirds. Journal of Experimental Biology 178:39–57.

Wethington, S. M., and S. M. Russell. 2003. The seasonal distribution and abundance of hummingbirds in oak woodland and riparian communities in Southeastern Arizona. Condor 105:484–495.

Weymouth, R. D., R. C. Lasiewski, and A. J. Berger. 1964. The tongue apparatus in hummingbirds. Acta Anatomica 58:252–270.

Williams, N. A. 1978. Hematozoa of wild birds from British Columbia. Journal of Parasitology 64:556–558.

Williams, R. A. J., K. Segovia-Hinostroza, B. M. Ghersi, V. Gonzaga, A. T. Peterson, and J. M. Montgomery. 2012. Avian influenza infections in nonmigrant land birds in Andean Peru. Journal of Wildlife Diseases 48:910–917.

Williamson, F. S. L. 1956. The molt and testis cycles of the Anna Hummingbird. Condor 58:342–366.

Williamson, S. 2001. A field guide to hummingbirds of North America. Houghton Mifflin Harcourt Publishing Company, New York, NY.

Wimsatt, J., N. Canon, R.D. Pearce, L.M. Vap, and M. David. 2009. Assessment of novel avian renal disease markers for the detection of experimental nephrotoxicosis in pigeons (*Columba livia*). Journal of Zoo and Wildlife Medicine 40:487–494.

Winker, K. 2000. Obtaining, preserving, and preparing bird specimens. Journal of Field Ornithology 71:250–298.

Winker, K., B. A. Fall, J. T. Klicka, D. F. Parmelee, and H. B. Tordoff. 1991. The importance of avian collections and the need for continued collecting. The Loon 63:238–246.

Withers, P. C. 1977a. Measurement of VO2, VCO2, and evaporative water loss with a flow through mask. Journal of Applied Physiology 42:120–123.

Withers, P. C. 1977b. Respiration, metabolism, and heat-exchange of euthermic and torpid poorwills and hummingbirds. Physiological Zoology 50:43–52.

Wolf, B. O., A. E. McKechnie, C. J. Schmitt, Z. J. Czenze, A. B. Johnson, and C. C. Witt. 2020. Extreme and variable torpor among high-elevation Andean hummingbird species. Biology Letters 16:20200428. https://doi.org/10.1098/rsbl.2020.0428

Wolf, L. L. 1969. Female territoriality in a tropical hummingbird. Auk 86:490–504.

Wolf, L. L., and F. R. Hainsworth. 1972. Environmental influence on regulated body temperature in torpid hummingbirds. Comparative Biochemistry and Physiology. Part A, Physiology 41:167–173.

Wolf, L. L., F. Reed Hainsworth, and F. Gary Stiles. 1972. Energetics of foraging: rate and efficiency of nectar extraction by hummingbirds. Science 176:1351–1352.

Wright, N. A., T. R. Gregory, and C. C. Witt. 2014. Metabolic 'engines' of flight drive genome size reduction in birds. Proceedings of the Royal Society B, Biological Sciences 281:20132780. https://doi.org/10.1098/rspb.2013.2780

Yamasaki, Y. K., E. E. Graves, R. S. Houston, B. M. Oconnor, P. E. Kysar, M. H. Straub, J. E. Foley, and L. A. Tell. 2018. Evaluation of *Proctophyllodes huitzilopochtlii* on feathers from Anna's (*Calypte anna*) and Black-chinned (*Archilochus alexandri*) Hummingbirds: prevalence assessment and imaging analysis using light and tabletop scanning electron microscopy. PLOS ONE 13(2):e0191323. https://doi.org/10.1371/journal.pone.0191323

Zamparo, D., D. R. Brooks, and D. Causey. 2003. *Whallwachsia illuminata* n. gen., n. sp. (Trematoda: Digenea: Plagiorchiformes: Prosthogonimidae) in the steely-vented hummingbird *Amazilia saucerrottei* (Aves: Apodiformes: Trochilidae) and the yellow-olive flycatcher *Tolmomyias sulphurescens* (Aves: Passeriformes: Tyraninidae) from the Area de Conservación Guanacaste, Guanacaste, Costa Rica. Journal of Parasitology 89:814–818.

Zenzal, T. J., Jr., R. H. Diehl, and F. R. Moore. 2014. The impact of radio-tags on Ruby-throated Hummingbirds (*Archilochus colubris*). Condor 116:518–526.

Zenzal, T. J., Jr., and F. R. Moore. 2016. Stopover biology of Ruby-throated Hummingbirds (*Archilochus colubris*) during autumn migration. Auk 133:237–250.

Zenzal, T. J., Jr., F. R. Moore, R. H. Diehl, M. P. Ward, and J. L. Deppe. 2018. Migratory hummingbirds make their own rules: the decision to resume migration along a barrier. Animal Behaviour 137:215–224.

Zenzal, T. J., and F. R. Moore. 2019. Resource use and defence by ruby-throated hummingbirds during stopover. Behaviour 156:131–153.

Addresses of authors:

LISA A. TELL

Department of Medicine and Epidemiology
School of Veterinary Medicine
University of California, Davis
Davis, CA 95616 USA
latell@ucdavis.edu

JENNY A. HAZLEHURST

California State University East Bay
Biological Sciences Department
Hayward, CA 94542 USA
jenny.hazlehurst@csueastbay.edu

RUTA R. BANDIVADEKAR

Department of Medicine and Epidemiology
School of Veterinary Medicine
University of California, Davis
Davis, CA 95616 USA
rbandivadekar@ucdavis.edu

JENNIFER C. BROWN

US Fish and Wildlife Service
Sacramento, CA 95825 USA
jennifercbrown@fws.gov

AUSTIN R. SPENCE

Department of Ecology and Evolutionary Biology
University of Connecticut
Storrs, CT 06269 USA
austin.reid.spence@gmail.com

DONALD R. POWERS

Biology & Chemistry Department
George Fox University
Newberg, OR 97132 USA
dpowers@georgefox.edu

DALEN W. AGNEW

Department of Pathobiology and Diagnostic Investigation
Michigan State University Veterinary Diagnostic Laboratory
College of Veterinary Medicine
4125 Beaumont Road
Lansing, MI 48910 USA
agnewd@msu.edu

LESLIE W. WOODS

Department of Pathology, Microbiology and Immunology
School of Veterinary Medicine
University of California, Davis
Davis, CA 95616 USA
and
California Animal health and Food Safety Laboratory
University of California, Davis
Davis, CA 95616 USA
lwwoods@ucdavis.edu

ANDREW ENGILIS, JR.

Department of Wildlife, Fish, and Conservation Biology
Museum of Wildlife and Fish Biology
University of California, Davis
Davis, CA 95616 USA
aengilisjr@ucdavis.edu